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Occlusive Vascular Diseases in Oral Contraceptive Users

Epidemiology, Pathology and Mechanisms

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Abstract

Despite being an unprecedented departure from normal physiology, the combined oral contraceptive is not only highly effective, but it also has a remarkably good safety record. Concerns over safety persist, though, particularly with regard to venous thromboembolism (VTE), stroke and myocardial infarction (MI). Epidemiological studies consistently show an increase in risk of VTE, but the results are more contentious with regard to arterial diseases. Despite 40 years of research, the mechanisms behind these adverse effects are not understood. In this review, we integrate information from published studies of the epidemiology and pathology of the occlusive vascular diseases and their risk factors to identify likely explanations for pathogenesis in oral contraceptive users. Oral contraceptives induce both prothrombotic and fibrinolytic changes in haemostatic factors and an imbalance in haemostasis is likely to be important in oral contraceptive-induced VTE. The complexity of the changes involved and the difficulty of ascribing clinical significance has meant that uncertainty persists. A seriously under-researched area concerns vascular changes in oral contraceptive users. Histologically, endothelial and intimal proliferation have been identified in women exposed to high plasma estrogen concentrations and these lesions are associated with thrombotic occlusion. Other structural changes may result in increased vascular permeability, loss of vascular tone and venous stasis. With regard to arterial disease risk, epidemiological information relating to dose effects and joint effects with other risk factors, and studies of pathology and changes in risk factors, suggests that oral contraceptive use *per se* does not cause arterial disease. It can, nevertheless, synergise very powerfully with subclinical endothelial damage to promote arterial occlusion. Accordingly, the prothrombotic effects of the oral contraceptive estrogen intervene in a cycle of endothelial damage and repair which would otherwise remain clinically silent or would ultimately progress – in, for example, the presence of cigarette smoking or hypertension – to atherosclerosis. Future work in this area should focus on modification of the effects of established risk factors by oral contraceptive use rather than modification of the supposed risk of oral contraceptive use by established risk factors. Attempts to understand vascular occlusion in oral contraceptive users in terms of the general features of VTE or with reference to atherosclerosis may be limiting, and future work needs to acknowledge that such occlusions may have unique features. Unequivocal identification of the mechanisms involved would contribute considerably to the alleviation of fears over vascular disease and to the development of even safer formulations.

The availability of estrogen/progestogen containing oral contraceptives since 1960 represents a major development in public health. The long term administration of powerful pharmacological agents to healthy young women to entirely impede a process which – in evolutionary terms – has been under continual optimisation for some considerable time is also an unprecedented departure, both from normal physiology and from traditional medical practice. In view of this, it is remarkable that steroidal contraception is as safe as it is. Nevertheless, concerns continue to be expressed over the health of women using this form of contraception.

The occlusive vascular diseases – venous thromboembolism (VTE), ischaemic stroke (haemorrhagic stroke will also be considered here) and myocardial infarction (MI) – are the most serious conditions that have been linked with oral contraceptive use. Substantial changes in oral contraceptive formulation and prescribing practice have

been made in response to these concerns. However, it should be appreciated that these changes were often made with only equivocal evidence for their efficacy or even for the existence of the risks they were intended to diminish.

Such uncertainty stems primarily from the exceptional difficulty of studying vascular disease in oral contraceptive users. The occlusive vascular diseases are extremely rare in young women so that, even if risk is increased, the absolute number of events is very small. Epidemiological investigations in this area must be either very large, or of extended duration. Evaluation of oral contraceptive-induced changes in metabolic, haemostatic and physiological risk factors has emerged as an alternative strategy with the potential for anticipating changes in risk, rather than assessing such changes after the disease event. However, this approach is still in a relatively early stage of development. Only hypertension and plasma low density lipoprotein (LDL) cholesterol levels have been sufficiently well investigated for there to be a consensus over their direct involvement in the development of cardiovascular disease, and for there to be interventions in place that have proved to be effective. The many other potential or putative risk factors that have been identified have yet to be so well researched.

Given these uncertainties, major changes in oral contraceptive formulation and prescribing practice may be questioned. However, any increased risk of vascular disease in healthy young women taking medication is seen as sufficiently unacceptable for suspicion and inference to be grounds for action. Unfortunately, this has made a 'moving target' of the oral contraceptive, with composition and usage often being changed before sufficient time has elapsed for effective epidemiological evaluation, or for the implications of oral contraceptive-induced changes in cardiovascular disease risk factors to be fully understood.

Uncertainties relating to epidemiologically-determined risks of vascular disease associated with oral contraceptive use and to the importance of oral contraceptive-induced changes in risk factors have been the main topics of debate over vascular disease in oral contraceptive users. However, this emphasis on epidemiology and risk factors has tended to obscure the important but seriously understudied area of vascular pathology. As will be shown, the few studies there are indicate that such pathology may show certain unique features, thus calling into question attempts to understand vascular disease in oral contraceptive users in terms of the more generally encountered vascular pathologies. Beyond even this uncertainty is the fact that although almost 40 years has elapsed since the first case was reported, there is still no accepted mechanistic explanation for the increased risks of venous thrombosis, MI or stroke that have been described in some oral contraceptive users.

Reviews of oral contraceptives and cardiovascular disease have generally focused on one single aspect, or have collated in one volume a brief overview and isolated summaries of different aspects. In view of the uncertainties that exist in both the epidemiological and risk factor information, we consider that a unifying approach may be more effective. Such an approach has been adopted in the recent report from the World Health Organization (WHO) Scientific Group,[1] but this concentrates mainly on the health implications of current oral contraceptive prescribing practice, and makes no mention of vascular pathology. We therefore consider in some detail both the historical and more recent literature on the epidemiology of vascular disease in oral contraceptive users. With this information as reference, we then assess the extent to which the occlusive vascular diseases, when encountered in oral contraceptive users, resemble or differ from the more generally observed conditions. We then assess the risk factor changes seen in oral contraceptive users and determine which changes are likely to be relevant. Finally, we include a section that collates information regarding the controversy over differences in risks of vascular disease between users of low estrogen dose oral contraceptives containing the progestogens desogestrel or gestodene and those containing levonorgestrel (the 'third versus second generation pill

controversy'). The original stimulus for assembling a review on this scale was the unexpected finding of a doubling in risk of VTE in users of the former compared with the latter. The debate over whether this difference was caused by the formulations concerned or was the consequence of study bias or confounding has continued since these findings were first made public in October 1995. Moreover, at the time of finalising this review (August 2000), these findings remain the subject of legal proceedings, regulatory agency appeals and continuing studies. In view of the continuing debate, and the fact that new, relevant study findings are likely to continue to appear for some years to come, we have chosen to assemble a single summary section which can act as a point of reference for the interpretation of new findings. Such an overview is particularly appropriate in the context of the overall aim of this work which has been to develop and identify coherent lines of evidence that can be used to support reference statements concerning associations between oral contraceptive use and cardiovascular disease, and possible mechanisms that might contribute to these associations.

1. Oral Contraceptive Steroids

The principal female sex hormones in premenopausal women are estradiol 17-β (estradiol) and progesterone. Therapeutically, synthetic gonadal steroids are used in preference to the native hormones mainly for their ease of administration and their higher biological activity. This is particularly important in oral contraception which requires that a given formulation be essentially 100% effective in all users. The estrogen component of current combined oral contraceptives is ethinylestradiol which is orally active and has an estrogenic potency about 100-times higher than that of the natural hormone.[2]

Progesterone is not used in oral contraceptive therapy because of difficulty of administration and adverse effects. Synthetic alternatives to progesterone have been employed which are variants on the structure of testosterone or progesterone and can have considerably higher progestogenic activity than progesterone itself. 19-nortestosterone and 17-hydroxyprogesterone are the principal parent compounds, giving rise to 3 series of progestogens: the gonane and estrane series, derived from 19-nortestosterone and having either an ethyl or a methyl substituent at the 13 carbon atom of the steroid nucleus, and the pregnane series, derived from 17-α hydroxyprogesterone.

The gonane series includes levonorgestrel, and the more recently introduced desogestrel, gestodene and norgestimate. These latter steroids are characterised by higher selectivity for progesterone rather than androgen receptors. The estrane series includes norethisterone, etynodiol (ethynodiol diacetate), noretynodrel (norethynodrel) and lynestranol. The pregnane series includes medroxyprogesterone, chlormadinone and megestrol.

The structures of the principal synthetic oestrogens and progestogens used in oral contraceptives and their parent compounds are shown in figure 1.

2. Epidemiology of the Occlusive Vascular Diseases in Oral Contraceptive Users

The epidemiology of vascular disease in oral contraceptive users has been reviewed frequently,[3-14] but there have been few attempts to place the epidemiological data in the context of pathology and pathogenesis. Epidemiological investigation of occlusive vascular disease in oral contraceptive users has been hindered by the rarity of vascular disease among women of childbearing age. The most common condition is VTE with an incidence among non-pregnant women, not using oral contraceptives of less than 11 per 100 000 women per year.[1,15] MI and stroke are considerably rarer. Epidemiological investigation of vascular disease among oral contraceptive users therefore involves the discrimination of differences in small numbers of events. For information to be in any way reliable, studies have to be large. According to previously published criteria for an ideal study,[11] case-control studies should include a predetermined method for selection of participants which should remain unmodified during the course of the study. In addition,

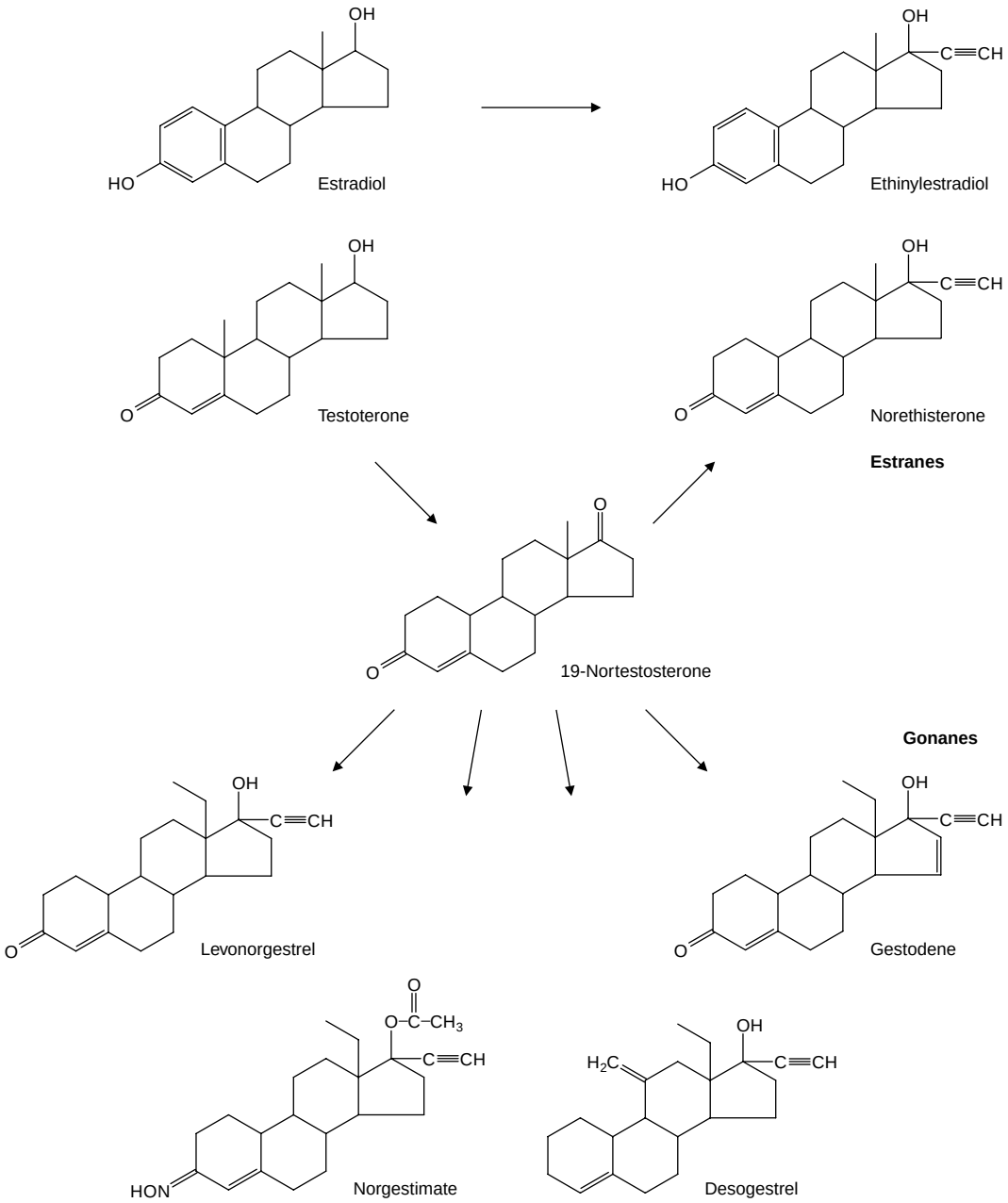

Fig. 1. Structures of synthetic gonadal steroids commonly used in oral contraceptives and relationships to their parent compounds.

there should be precise definition of oral contraceptive exposure; interviewers should not be aware of a person's status as case or control; interviewers should not be aware of the study hypothesis; only physicians' records should be used to assess oral contraceptive exposure; cases and controls should have undergone the same diagnostic examinations and procedures; oral contraceptive users and non-users should have been under equal medical surveillance; and cases and controls should have equal clinical susceptibility. With regard to cohort studies, there should be randomisation of individuals to oral contraceptive user and non-user groups, exposure to oral contraceptives should be continually monitored during follow-up, oral contraceptive users and non-users should undergo equal medical surveillance and oral contraceptive users should be followed from their initial exposure to oral contraceptives.

These criteria serve primarily to highlight the difficulties in studying vascular disease among oral contraceptive users. In practice, almost all conceivable sources of information have been used to either investigate or support conclusions regarding vascular disease in oral contraceptive users. Sources of data have included family practitioner, pharmacy, hospital admission and hospital discharge records, case reports, death certificates, postal questionnaires, planned follow-up of population samples, general practice databases and national mortality statistics. Rather than trials being randomised, studies have been almost exclusively observational. The mainstay of investigation has been the case-control study, which has the advantage of enabling identification of substantial numbers of cases within a relative short timeframe and which, with sufficient numbers, can allow for effective investigations into and adjustment for the influence of confounding factors. Of the many case-control studies of vascular disease in oral contraceptive users, the largest has been The WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception (the WHO Collaborative Study).[16] This was a hospital-based, case-control study (with community controls from one centre) of nonfatal VTE, MI, and ischaemic and haemorrhagic stroke. 21 centres throughout the world recruited a total of 3276 cases and 8811 controls between the years 1989 and 1993.

With the exception of the very first clinical evaluation of oral contraceptives carried out in Puerto Rica,[17,18] there has not been a randomised, placebo-controlled trial of the effects of oral contraceptives on occlusive vascular disease. For ethical and practical reasons, it is now inconceivable that there ever will be such a trial. Moreover, participants in such a trial would be likely to differ from those exposed to comparable treatment in the general population on account of their compliance and exposure to medical attention. Studies of vascular disease in oral contraceptive users are, therefore, necessarily observational and, as such, are susceptible to bias and confounding. For example: vascular disease events reported to an investigator may be more likely to involve oral contraceptive users; interviewers might probe more deeply for a history of oral contraceptive use in cases rather than controls; there may be a higher index of suspicion for vascular disease in women using oral contraceptives, who are therefore referred to hospital more readily for investigation; and patients with a condition linked with oral contraceptive use may be more likely to recall use of oral contraceptives. Finally, hospital and community-derived controls may differ systematically in their exposure to oral contraceptives, and hospital controls may also differ from cases recruited in hospital. Since women do not choose to use oral contraceptives randomly, there may be confounding by characteristics linked to both the disease in question and the woman's status as an oral contraceptive user. Statistical techniques may be used to correct for the influence of such factors, but these do not necessarily eliminate the effect in question, particularly when the confounding influence is widely prevalent.[14] It is more satisfactory to undertake analyses in separate strata of the confounder in question, and examine the modification in oral contraceptive effect across such strata. However, this can only be done effectively in the larger studies. Issues of study design and effect

modification in epidemiological investigations of vascular disease in oral contraceptive users have recently been reviewed in detail.[14,19]

Although the bulk of recent epidemiological information on vascular disease in oral contraceptive users comes from case-control studies, several influential cohort studies have been undertaken, primarily during the 1970s. Since these have each contributed information on all the different vascular diseases in question (primarily in association with use of oral contraceptives containing ethinylestradiol 0.05mg) they are summarised briefly here.

The Royal College of General Practitioners' (RCGP) Oral Contraception Study described itself as '...a controlled investigation of the natural history of a large group of women (initially 46 000) half of whom had chosen the pill as a contraceptive'.[20] Throughout 1968 and 1969 1400 UK general practitioners identified 23 000 users and 23 000 non-users for long term observation. By October 1987, about 65% of the original cohort had been lost to follow-up.[21] Compared with non-users, users had more children, smoked cigarettes more frequently, and were less likely to have had health problems. This study was informative with regard to the possible influence of additional medical surveillance on disease incidence in oral contraceptive users: 18% of diagnoses of disease of any kind in participants in this study were made on the same date as an oral contraceptive prescription was issued, and overall reporting of morbidity was 19% higher in users compared with non-users or past-users. These figures indicate the likely upper limit of magnitude in any bias resulting from the increased medical attention which the oral contraceptive users might have received.

The second major cohort study, which began at the same time as the RCGP study, was the Oxford/Family Planning Association (FPA) Study.[22] Whereas the RCGP study depended on the general practitioner system for follow-up and surveillance, the Oxford/FPA Study took advantage of an established family planning clinic network. Between 1968 and 1974, 17 clinics recruited 17 032 women. This study had the advantage of recruiting a comparison group using nonsteroidal contraception. At the time of the first interim report, 56 000 women-years of observation had accumulated, with an annual loss to follow-up of only 0.3%.[22] The RCGP study provided information primarily about risks associated with current oral contraceptive use, although some information regarding past use was also available. This was also a feature of the Oxford/FPA Study, in which associations between past use and vascular disease in women who had stopped taking oral contraceptives because of health problems were particularly important. In contrast, the Nurses' Health Study,[23] a prospective study of women in the US, was mainly concerned with effects in past users. This cohort was established in 1976 when questionnaires were mailed to 121 700 female nurses aged 30 to 55 years. This age range meant that most of the clinical information gathered in subsequent 2-yearly follow-ups related to women who had ceased using oral contraceptives. This survey included 62 718 who had never used oral contraceptives, 49 269 who had used them in the past and 7074 current users. A further US cohort study, the Walnut Creek Contraceptive Drug Study,[24] although large (about 110 000 women-years of follow-up), provided less useful information as the mean duration of follow-up was only 6.5 years. During this period there were, for example, only 26 reports of MI, with relatively few events in women of reproductive age.

2.1 Venous Thromboembolism (VTE)

Inaccurate diagnosis has been a problem in studies of the epidemiology of VTE, particularly when such diagnoses were made on purely clinical grounds.[25,26] However, confirmation of diagnosis by venography, duplex scanning or radioactive fibrinogen uptake studies has been a feature of more recent studies. Confirmed diagnosis of pulmonary embolism requires ventilation/perfusion scanning or angiography.

2.1.1 Epidemiology of VTE in Young Women

The incidence of VTE in young women with no known risk factors for thrombosis has been best

quantified in cohort studies of VTE in oral contraceptive users or in analyses of national diagnosis registers. Recent estimates have been in the region of 3 to 11 per 100 000 women per year.[15,27-29] With an estimated case fatality rate of 1 to 2%, this represents about 1 death per 2 million women who are not known to be predisposed towards the condition. On the basis of these figures, about 6 deaths per year from VTE not associated with predisposing conditions would be expected in the UK, for example, among younger women. Overall, death from VTE among women in the age range 15 to 49 years is currently estimated to be 78 deaths per year[30] so the great majority of deaths must be associated with the known predisposing conditions for the disease.

According to estimates based on WHO statistics for developed countries in the period 1992 to 1996,[1] the estimated number of thromboembolic events among women not taking oral contraceptives in the age ranges 20 to 24, 30 to 34 and 40 to 44 years were 32, 46 and 59 per million per year. Equivalent figures for death from VTE were 0.6, 0.9 and 1.2 per million per year. The system employed in Denmark whereby all hospital admissions are recorded on a central database (the National Patient Register), and annual mortality statistics (the Danish Death Statistics) are made available by the Danish National Board of Health, make estimates of both national morbidity and national mortality in this country particularly reliable. Incidence of and death from VTE in 5-year age bands in women in Denmark between the ages 15 and 44 years in the period 1980 to 1993 are illustrated in figure 2.[29] The estimated number of thromboembolic events (excluding pregnant women) in the age ranges 20 to 24, 30 to 34 and 40 to 44 were 110, 167 and 301 per million per year. Equivalent figures for death from VTE were 0.8, 0.7 and 8.9 per million per year.

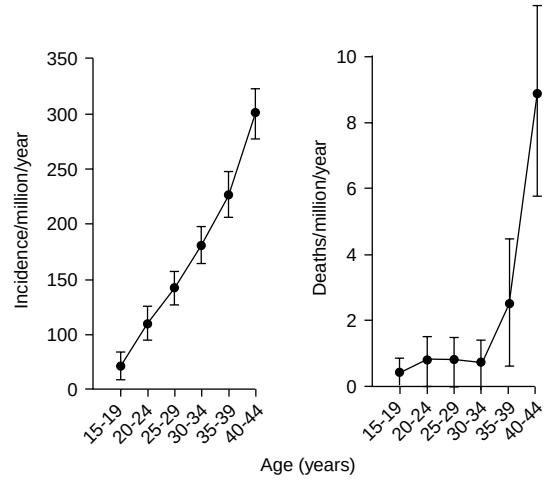

Fig. 2. Morbidity (n = 2653) and mortality (n = 42) for venous thromboembolism in young women in Denmark from 1980 to 1993, excluding pregnant and puerperal women (rate point estimates and 95% confidence intervals are shown) [reproduced from Lidegaard,[29] with permission].

2.1.2 Risk Factors for VTE in Young Women

The risk of VTE is elevated in association with conditions such as obesity, pregnancy, surgery, malignant disease and immobilisation. A previous thrombotic episode increases the likelihood of a further thromboembolic event. Evidence regarding risk factors for VTE that relates specifically to young women comes largely from analyses of confounder effects and risk factor variations in studies of VTE in oral contraceptive users. For example, in the RCGP study no association was found between the incidence of deep vein thrombosis (DVT) and age, social class or parity, although there was a strong correlation between superficial phlebitis and thrombophlebitis and age.[20] It should be noted that the lack of association between age and DVT in this study may reflect the confounding effect of pregnancy: other studies, which have discriminated between pregnant and non-pregnant women have found a strong positive association between age and risk of DVT.[29,31] The WHO Collaborative Study found no differences in number of live births, marital status, cigarette smoking or alcohol consumption between cases of VTE and controls, but cases did have a significantly higher mean body mass index (BMI).[32] A difference in the pattern of risk factors between European women and those in developing countries (such as Africa, Asia and Latin America)

was also noted, with cases in Europe being less likely to have entered into further education and more likely to have developed hypertension in pregnancy than their controls.[32] Cases in developing countries were more likely to have had at least one live birth, a history of hypertension and rheumatic heart disease, and a family history of premature cardiovascular disease than their controls. Crude odds ratios for VTE associated with a range of potential risk factors in the WHO Collaborative Study are shown in table I. The lack of association between cigarette smoking and VTE in this study has been confirmed in other studies in young women,[20,33] although a recent analysis of a UK general practice database suggests that there may be some association.[34]

With regard to hereditary factors in VTE, the Leiden Thrombophilia study found a relative risk (RR) of 2.9 [95% confidence interval (CI) 1.6 to 5.1] associated with a family history of venous thrombosis in women aged 15 to 49 years.[35] This study also reported an 8-fold increase in risk associated with the factor V Leiden mutation which confers resistance to the action of activated protein C (APC). Family history was also a significant factor in the study of Danish hospital records, with an odds ratio (OR) of 3.2 (95% CI 2.1 to 4.7) for VTE in those with a family history relative to those without.[31]

2.1.3 VTE in Oral Contraceptive Users

It has been suggested that if a woman is known to be an oral contraceptive user and presents with symptoms compatible with VTE, the physician, aware that oral contraceptive use carries an increased risk, may be more inclined to diagnose VTE on purely clinical grounds, or refer the woman for hospital investigation. In the RCGP Study, a marked differential in risk of deep vein and superficial vein thrombosis among oral contraceptive users was taken as evidence against the operation of such diagnostic bias. Furthermore, there was no variation in diagnosis rates as a result of the serious public concern that developed over risk of VTE following December 1969, when the UK Committee for the Safety of Drugs recommended against the use of oral contraceptives containing more than 0.05mg estrogen – risks of VTE were similar in magnitude to those that had been detected before this date. In the WHO Collaborative Study, 3 categories of diagnosis were distinguished: definite, probable and possible.[32] If referral or diagnostic bias was operating, it might be expected that higher odds ratios for VTE in oral contraceptive users would be found in the probable or possible categories. However, this was not the case. Any uncertainty in diagnosis appeared to be associated with lower risks, as would be expected from diagnostic imprecision rather than bias. There was evidence in this study for investigation bias, whereby suspected DVT is more rigorously investigated in oral contraceptive users than in non-users. The authors speculated that this might have resulted from greater case-severity in oral contraceptive users, but this would have led to higher odds ratios for venous thrombosis in oral contraceptive users in the definite compared with probable diagnosis categories, and, in any case, this differential was not apparent for pulmonary embolism.

Table I. Relative risks (±95% confidence intervals) of venous thromboembolism (VTE) associated with potential risk factors for VTE in the World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception[32] (family history of VTE was not reported in this study)

Risk factor	Europe	Developing countries
History of rheumatic heart disease	2.3 (0.7-7.6)	33.0 (4.3-255.6)
BMI >30kg/m^2 (*vs* ≤20kg/m^2)	2.7 (1.7-4.3)	4.6 (2.8-7.7)
History of varicose veins	2.7 (1.7-4.1)	3.8 (2.8-5.3)
≥10 cigarettes per day (*vs* non-smokers)	2.6 (0.5-14.6)	1.2 (0.1-13.5)
Hypertension in pregnancy	1.7 (1.2-2.3)	1.2 (0.9-1.6)
History of hypertension	1.0 (0.6-1.6)	1.8 (1.3-2.7)

Table II. Case-control studies of venous thromboembolism in oral contraceptive (OC) users

Date and source of cases	No. of cases (OC users)	Odds Ratio	Reference
1961-66 GP records; nonfatal DVT and PE	24 (5)	2.3 (0.6-8.6)	38
1963-67 US hospital records; postoperative or post-trauma DVT or PE	60 (?13)	6.5 (1.6-27.0)	39
1964-67 UK hospital records; nonfatal idiopathic DVT or PE	84 (42)	6.3 (3.5-11.3)	33, 40
1964-67 UK hospital records; nonfatal postoperative DVT or PE	30 (12)	3.8 (1.4-10.2)	41
1965-67 US hospital records; nonfatal DVT and PE	175 (67)	4.4 (2.3-8.4)	42, 43
1964-68 Swedish hospital admissions; nonfatal idiopathic DVT	84[a]	11 (?)	44
1966 UK death certificates; fatal idiopathic PE	26 (16)	8.3 (4.2-16.5)	45
1969-74 US hospital admissions; cohort study, idiopathic VTE or PE	17 (8)	7.7 (2.9-20.7)	46
1972 US hospital admissions; nonfatal idiopathic DVT	43 (31)	11.0 (5.2-25)	47
1970-73 US hospital admissions; nonfatal idiopathic VT or	92 (?)	5.0 (2.4-10.3)	48, 49
PE ?; non-fatal DVT and PE	61 (20)	8.1 (3.7-18)	50
1986-88 UK death certificates; idiopathic, fatal DVT and PE	41 (22)	2.1 (0.8-5.2)	51
1989-93 European hospital admissions; nonfatal idiopathic VTE or PE	433 (265)	4.1 (3.1-5.6)	32
1989-93 Developing country hospital admissions; nonfatal idiopathic VTE or PE[b]	710 (205)	3.3 (2.6-4.1)	32
1991-95 European hospital admissions; nonfatal idiopathic VTE or PE	505 (334)	4.4 (3.4-5.8)	52
1994-95 All Danish hospital admissions; nonfatal idiopathic VTE or PE	375 (172)	2.1-5.1[c]	31

a National prescribing records used as control.

b Developing countries: Jamaica, Kenya, Zimbabwe, China, Hong Kong, Indonesia, Thailand, Brazil, Chile, Colombia, Mexico.

c Odds ratios with duration of use decreasing from >5 years to <1 year – all significant.

DVT = deep vein thrombosis; **GP** = general practitioner; **PE** = pulmonary embolism; **VTE** = venous thromboembolic event; **?** = unknown.

Both referral and diagnostic bias were minimised in the study of Bloemenkamp et al.[36] in which increased odds ratios for VTE in oral contraceptive users were found among women limited to those referred to specialist centres for suspected VTE. Diagnostic bias was minimised in these specialist centres by the routine use of rigorous diagnostic procedures and, since only those with suspected venous thrombosis were considered, it was argued that referral bias was eliminated. This study design does not control for differentials in cases of suspected VTE which subsequently proved positive between oral contraceptive users and non-users. It appears more likely, however, that any bias arising from such variations would tend to minimise the apparent risk in oral contraceptive users, since those not taking oral contraceptives and suspected of having a thrombosis could be more likely to be genuine cases.

Case-Control Studies

The first report linking idiopathic VTE to oral contraceptives was a case history that appeared in the Lancet in 1961.[37] Further reports followed, and stimulated the numerous case-control studies of nonfatal and fatal VTE that have been published since (table II).

Overall, case-control studies comparing VTE in current users and non-users indicate an increase in risk of between 4 and 8, the greatest relative risk reported being 11. Of particular interest are 2 large studies which have examined the risk of VTE in women using oral contraceptives primarily during the early 1990s.[32,52] Between February 1989 and January 1993, the WHO Collaborative Study identified 433 first cases of DVT or pulmonary embolism among women aged 20 to 44 years in European countries and 710 cases in non-European ('developing') countries.[32] Cases were categorised according to definite, probable and possible diagnosis. An average of 2.4 controls per case were recruited in Europe and 2.8 in developing countries. Odds ratios for VTE in current oral contraceptive users relative to non-users were 4.2 (95% CI 3.1 to 5.6: adjusted for hypertension in pregnancy) and 3.3 (2.6 to 4.1) for European and developing coun-

tries, respectively. The second study came from the Transnational Research Group on Oral Contraceptives and the Health of Young Women (The Transnational Study), established to investigate the apparent excess of case reports of VTE among users of gestodene-containing oral contraceptives in Germany.[52] The study specifically employed the protocol used in the WHO Collaborative Study. The final analysis from the Transnational Study included the 505 cases of VTE from Germany and the UK up to December 1995.[53] Use of any oral contraceptive compared with non-use had an relative risk of 4.4 (3.4 to 5.8) for VTE, a figure closely in line with that found in the WHO Collaborative study.

Cohort Studies

A number of cohorts have been used to assess the risk of VTE in oral contraceptive users, including the major cohort studies described in the introduction to section 2. Risk ratios have varied between 0.3 and 7.2, but those larger studies, which considered all idiopathic VTE, generally gave risk ratios in excess of 2 for current use (table III).

Joint Effects with Other Risk Factors

With some exceptions,[42,48] there does appear to be a tendency for oral contraceptive users who develop VTE to be overweight. This was seen in the study of Vessey and Doll,[33,40] in the study of postoperative VTE of Vessey et al.[41] and in the analysis of predisposed cases of Greene and Sartwell.[39] In the WHO Collaborative Study, oral contraceptive users with high BMI had a higher odds ratio for VTE in both Europe and developing countries. Among Europeans, oral contraceptive users with BMI $\leq 25 kg/m^2$ had an odds ratio of 3.9 whereas obese users had an odds ratio of 7.0. This trend was also apparent in the recent study of Danish hospital admissions in which the following trend was observed relative to those with a BMI of $\leq 20 kg/m^2$: BMI 21-25kg/m^2, OR 1.2; 26 to 30kg/m^2, OR 1.9; $>30 kg/m^2$, OR 3.7.[31]

In contrast to MI (see section 2.2) there appears to be little effect of cigarette smoking on the risks of VTE associated with oral contraceptive use.[20,32,69] The WHO Collaborative Study found no relationship between risk of VTE, current oral contraceptive use and history of hypertension.[32] Among European women, risks of VTE associated with oral contraceptive use were higher among those with a history of hypertension during pregnancy. The odds ratio associated with such a history alone was 1.8, for oral contraceptive use alone 4.0, and 9.2 for both.

Among oral contraceptive users with factor V Leiden mutation, the odds ratio for VTE was appreciably greater (OR = 34.7) than among those with factor V Leiden mutation alone (OR = 7.9) or for oral contraceptive use alone (OR = 3.8).[35] Approximately similar relative risks associated with oral contraceptive use were found in the absence (RR = 3.7) and in the presence (RR = 5.0) of the mutation, and in logistic modelling of the risks associated with oral contraceptive use and factor V Leiden mutation, a multiplicative interaction term comprising the two risks was associated with no independent risk beyond that associated with the two factors separately. Similarly, risk of VTE associated with oral contraceptive use among women homozygous for the factor V Leiden mutation was found to be the same as for women with other genotypes.[70] However, the very high risk associated with the homozygous state was multiplied 4- to 6-fold by the risk associated with oral contraceptive use, resulting in very high risks indeed associated with the combination of homozygosity for factor V Leiden mutation and oral contraceptive use. Using population incidence data, rates of 0.8, 3.0, 5.7 and 28.5 per 10 000 women per year were estimated for factor V Leiden negative women not using and using oral contraceptives, and factor V Leiden positive women not using and using oral contraceptives, respectively.[35] These estimates demonstrated the considerable excess in cases associated with the combination of oral contraceptive use and factor V Leiden mutation. Confirmation of these finding comes from a recent case-control analysis of 148 women with a first, confirmed episode of venous thrombosis.[71] The odds ratio for venous thrombosis and oral contraceptive use in the absence of known genetic risk factors was 4.6

Table III. Prospective studies of venous thromboembolism in oral contraceptive (OC) users

Date	OC Status	Women-years of follow-up	No. of cases	Relative risk (95% confidence interval)	Reference
Maternal Health Project; Puerto Rico					
1961-69	Never users	17 353	1 fatal PE		
	Current users	12 155	0	-	17, 18
1961-69	Never users	13 874	8 thrombophlebitis	1.5 (0.6-3.8)	
	Current users	10 468	9		54
UK Royal College of General Practitioners Study					
1968-72	Never users	41 170	7 idiopathic leg DVT	5.6	55
	Current users	32 850	33		
1968-76	Never users	47 084	9 idiopathic leg DVT	4.2 (2.1-10.9)	56
	Current users	34 482	30		
1968-76	Never users	91 521	0 fatal PE		57
	Current users	91 880	1	-	
1968-79	Never users	138 630	0 fatal PE		58
	Current users	98 997	3	-	
Oxford/Family Planning Association Study					
1968-74	Never users	34 035	10 VTE or PE		22
	Current users	21 794	24	3.2	
1968-74	Never users	41 765	6 idiopathic VTE		59
	Current users	31 889	28	6.2	
1968-80	Never users	62 532	0 fatal PE		60, 61
	Ever users	79 678	0		
1968-87	Never users	118 671	x VTE[a]		62
	Ever users	152 597	29-x[a]	7.2	
Walnut Creek Contraceptive Drug Study					
1968-77	Never users	37 913	20 PE		24
	Current users	20 134	3	0.6 (0.2-1.6)	
Puget Sound Health Cooperative Study					
1977-82	Non-users	245 567	3 idiopathic VTE		27, 63
	Current users	54 971	7	2.8 (0.9-8.2)	
General Practice Research Database (365 UK general practices)					
1991-94	Past users	130 590	5 idiopathic VTE		28
	Current users	323 888	75	6.1 (unadjusted rate ratio)	
MediPlus Database (398 UK general practices)					
1991-95	Non-users	542 906	62 idiopathic VTE		15
	Current users	101 766	31	2.7 (unadjusted rate ratio)	
Other studies					
Munich clinic population					
?	Non-users	2640	1 idiopathic DVT	3.3 (0.4-27.7)	64
	Users	2783	3		
Australian general practitioners' survey					
1969-70	Non-users	1254	29 VTE	0.3 (0.2-0.7)	65
	Users	1044	8		
Postoperative DVT					
	Non-users		0 postoperative DVT		66
	Current users		6	-	

Table III. Contd

Date	OC Status	Women-years of follow-up	No. of cases	Relative risk (95% confidence interval)	Reference
US hospital admissions					
	Never users	n = 46 141	?	1.5 (age 25-50))	67
	Ever users	n = 19 682	?	5.0 (age 25-29)	
US clinical practices 1973-75					
	Never users	?	6 DVT or PE		68
	Current users	9621	11	1.1	

a 29 cases of VTE altogether, x in never users and 19-x in ever users.

DVT = deep vein thrombosis; **PE** = pulmonary embolism; **VTE** = venous thromboembolic event; **?** = unknown.

(95% CI 2.6 to 8.0), for the presence of factor V Leiden mutation in the absence of oral contraceptive use 2.4 (0.4 to 15.1) and for factor V Leiden mutation and oral contraceptive use together 20.0 (4.2 to 94.3). In this study, risks associated with a prothrombin gene mutation were also considered. The corresponding odds ratios for VTE and the prothrombin gene mutation (G20210A) were 2.7 (0.6 to 12.7) in the absence of oral contraceptive use, and 16.3 (3.4 to 79.1) for the prothrombin gene mutation and oral contraceptive use together (fig. 3). Consideration of the effects of risk factors in combination is of interest for the possible insights that may be gained into the mechanisms underlying the effects of the factors. For example, if the pathogenesis of VTE, is conceived as resulting from an imbalance developing in the continual cycle of fibrin generation and dissolution, the combination of a failure in the mechanisms that moderate thrombin generation (resulting from factor V Leiden mutation) and, say, a failure in the profibrinolytic mechanisms (resulting from oral contraceptive use) could accelerate progression to the development of an occlusive thrombus.

Effects of Duration of Current Oral Contraceptive Use

Studies of the effects of duration of use on risk of VTE in oral contraceptive users have generally been designed to explore whether or not thrombotic events become more common with increasing rather than decreasing duration of use. Vessey and Doll[33] found no consistent trend in their analysis of cases reported between 1964 and 1967 and noted that risk did not appear to be any greater in the first months of therapy. A similar lack of trend was apparent in the RCGP study report on cases accumulated between 1968 and 1972.[20] Detailed analyses of duration of use of oral contraceptives and risk of VTE were undertaken in the case-control studies from Sartwell et al.[42] and the Boston Collaborative Drug Surveillance Program[47] and in neither study was any association apparent, either in short or long term users.

More recent studies strongly suggest that there is an increased risk in the short term among younger women using oral contraceptives for the first time.[28,31,52,72-75] This would be consistent with the existence of a subset of women who are particularly susceptible to venous thrombosis on initial exposure to elevated concentrations of steroid hormones. This possibility has assumed importance in the controversy over differences in risks of VTE between users of low estrogen dose desogestrel- or gestodene-containing oral contraceptives and equivalent dose levonorgestrel-containing formulations, and the evidence is considered in detail in section 5 of this review.

Effects of Past Use of Oral Contraceptives

Studies have consistently found no evidence of a residual risk of VTE in current non-users compared with never users, in contrast to the significantly increased risks that were found in current users of oral contraceptives.[20,32,43,50,51] Progressive cessation of use of oral contraceptives in a population of women may, however, result in selection of current users who are less susceptible to oral contraceptive-associated risk of venous thrombosis.[73]

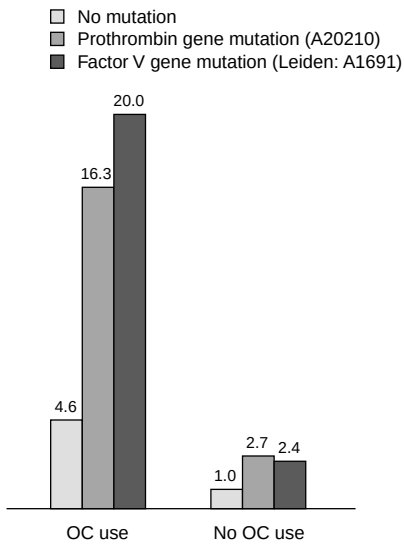

Fig. 3. Risks of venous thromboembolism (shown as odds ratios on each column) according to oral contraceptive use (OC) and the presence or absence of mutations in common factors of the haemostatic system known to affect thrombosis risk.[71]

Variation in Risk with Oral Contraceptive Formulation

A fundamental concern in assessing the cardiovascular risks associated with different oral contraceptives is that these may be seriously confounded by differences in the characteristics of the women who use different formulations. For example, there has been an active promotion of lower-dose formulations ever since the first studies in the late 1960s suggested that risk of VTE was increased in users of formulations containing more than 0.05mg estrogen compared with those containing 0.05mg or less (see next section). Therefore, at any given time beyond 1970, women still using a higher dose formulation from the range available will represent a selected group, usually older, who are satisfied with these earlier higher dose formulations. Equally, users of newly-introduced formulations are likely to be women just beginning oral contraception or women who might be considered to be at risk of VTE. Evidence for variation in risk according to formulation should therefore be carefully assessed with regard to possible confounding factors.

Oral Contraceptive Estrogen Content and VTE

Early studies of the association between oral contraceptive use and VTE found an overall excess of risk at all doses, but evidence of a trend towards decreasing incidence of the disease with decreasing oral contraceptive estrogen content (0.15 to 0.05mg).[32,42,45] Numbers of cases in some categories of estrogen dose were low, however, but overall the findings supported the existence of a small differential in risk between high and low dose formulations. Confirmation of this differential came in 1970 with the study of Inman et al.[76] where observed to expected ratios according to oral contraceptive estrogen dose were: 0.15mg (12 cases) 1.83; 0.1mg (63 cases) 1.04; 0.075 to 0.08mg (8 cases) 0.81; 0.05 to 0.06mg (50 cases) 0.89.

These findings emerged at a time when increased risk of VTE with estrogen therapy was being noted in other areas, including suppression of lactation with estrogens, prevention of coronary artery and cerebrovascular disease in men, and treatment of prostatic cancer. A climate of opinion existed therefore in which evidence for a link between VTE and higher oral contraceptive estrogen doses would be highly influential. Such evidence was provided by the report to the UK Committee on Safety of Drugs by Inman et al.[76] in which differences in risk associated with different oral contraceptive formulations were assessed on the basis of case reports, received by drug safety committees in the UK, Sweden and Denmark between 1965 and 1969. 920 reports from the UK concerning all types of combined oral contraceptive formulation in use over the specified period were analysed with respect to the influence of estrogen dose and progestogen content on risk of VTE. Fatal and nonfatal pulmonary embolism, deep vein and other thrombosis of the lower limb, all VTE, cerebral thrombosis and coronary thrombosis were each considered separately. Doses of mestranol were 0.05, 0.075 to 0.08, 0.1 and 0.15mg, and of ethinylestradiol 0.05 and 0.1mg. The observed number of reports compared with the expected number was higher for the highest dose of estrogen than for the lowest dose of estrogen in almost all categories for

both types of estrogen, a trend which was statistically significant. For example, fatal pulmonary embolism (which would have been diagnosed with some certainty), showed the following observed to expected ratios according to oral contraceptive estrogen dose: 0.15mg (6 cases) 2.79; 0.1mg (32 cases) 1.53; 0.075 to 0.08mg (7 cases) 0.96; 0.05mg (1 case) 0.56. However, these findings were not free from confounding by the progestogen component which varied between oral contraceptive types, no series of formulations having the same progestogen content but varying estrogen content.

Studies of adverse reaction reports have been criticised, although such criticisms have been addressed in detail.[76,77] One major consideration is that safety committees receive reports on only a fraction of the total number of adverse events occurring in association with oral contraceptive use – perhaps only 15% for fatal events – and there is a bias towards reporting of more recently introduced formulations.[45,78] Submission of reports to the committee may be substantially affected by the level of awareness of potential problems on the part of doctors. This may vary considerably according to the time since a formulation was first introduced, awareness decreasing as the time elapsed increases. Furthermore, this approach precludes any comparison with a control group of women not taking oral contraceptives. Instead, reference information is provided by national oral contraceptive sales figures, derived mainly from market research organisations and manufacturing companies. Nevertheless, conclusions based on adverse reaction reports have been highly influential in issues of oral contraceptive safety.

With regard to studies other than those based on adverse reaction reports, in the first report from the RCGP study,[20] the relative risk of all venous thromboses was evaluated with women taking formulations containing 0.05mg estrogen acting as reference group. Relative risk was 1.59 for women taking formulations containing estrogen 0.075 or 0.08mg and 1.61 for women taking formulations containing 0.1 or 0.15mg. No association with estrogen dose was found when the analysis was restricted to DVT, but with superficial vein thrombosis there was a positive association. Idiopathic VTE was evaluated in relation to estrogen dose by Stolley et al.[49] The risk relative to non-users was 4.7 (95% CI 2.7 to 8.2) among women taking formulations containing less than 0.1mg estrogen and 10.1 (4.7 to 21.7) among women taking formulations containing 0.1mg or more of estrogen.

More recently, the Michigan Medicaid cohort study found a positive relationship between estrogen potency and venous thrombosis (low, intermediate, high: RRs 1.0, 1.4, 2.6, respectively) and weaker positive relationship between estrogen dose and VTE risk (for dose of <0.05, 0.05, >0.05mg, RR was 1.0, 1.5, 1.7, respectively).[79,80] Such a relationship was not found in the studies of Helmrich et al.[50] Among recent case-control studies, the WHO Collaborative Study found a non-significant tendency towards higher odds ratios for VTE among users of formulations containing ≥0.05mg ethinylestradiol in all groups compared except European women under age 35 years.[32] Effects of estrogen dose in the WHO Collaborative Study may have been diluted by the increased incidence of VTE seen in users of very low estrogen dose formulations, which may be due to selective prescribing (see section 5). Similarly equivocal relationships were seen in the Danish hospital records study[31] and the Transnational Study.[53]

The diminution in effect of estrogen dose in recent studies, accords with the possibility that women still using the higher dose formulations are a self-selected group of long term oral contraceptive users who have found these formulations satisfactory. In accord with this, in a recent analysis from the Transnational Study[53] in which full oral contraceptive exposure history was incorporated into the analysis, the adjusted hazard ratios for VTE were 8.5 (95% CI 3.0 to 23.9) for formulations containing ≥0.05mg estrogen, 3.7 (2.2 to 6.1) for a formulation containing 0.035mg estrogen, 2.6 to 2.9 (1.5 to 4.0) for formulations containing 0.03mg estrogen and 1.6 (0.9 to 2.9) for a formulation containing 0.02mg estrogen.[81]

In conclusion, there does appear to be a relationship between risk of VTE and oral contraceptive estrogen dose over the broad range of doses that have been used since their introduction. The magnitude of this variation is difficult to quantify. In any case, the range of currently employed estrogen doses has, appropriately, been reduced towards the minimum necessary for effective contraception. These doses still appear to increase the risk of VTE. Future measures to reduce this risk should focus on individual susceptibility to VTE or the use of different steroid regimens, rather than further reductions in dose.

Progestogen Dose and VTE

In the analysis of adverse reaction reports by Inman et al.[76] published in 1970, two series of formulations were considered in which the estrogen dose was constant and the progestogen content varied. In one series, 5 formulations contained ethinylestradiol 0.05mg; in the other series, 3 formulations contained mestranol 0.1mg. Four of the 5 formulations included in the former series contained the same progestogen, norethisterone acetate, at doses 1.0, 2.5, 3.0 and 4.0mg. Observed to expected ratios for pulmonary embolism were (numbers of events reported are given in brackets) 1.02 (7), 0.67 (5), 0.55 (35) and 0.41 (12), respectively. For coronary thrombosis, the equivalent figures were 0.68 (1), 0.66 (1), 0.94 (12) and 1.09 (7). These analyses were extended by Meade and colleagues[77] who included all adverse reaction reports received between 1964 and 1977. The only formulations considered were those that could provide for analyses of progestogen effects, since low and high dose estrogen formulations differed entirely in progestogen content. The total number of reports considered was 2044; 122 exclusions were made on the basis of predisposing conditions, or uncertainty over diagnosis or oral contraceptive exposure. Six oral contraceptive formulations were considered, 4 containing norethisterone acetate at differing doses in the presence of ethinylestradiol 0.05mg, and 2 containing levonorgestrel at differing doses in the presence of ethinylestradiol 0.03mg. Observed to expected ratios for pulmonary embolism for the ethinylestradiol 0.05mg series containing norethisterone acetate at doses of 1.0, 2.5, 3.0 and 4.0 mg. were (number of events) 1.12 (135), 1.04 (16), 1.01 (135) and 0.56 (22), respectively. For ischaemic heart disease the equivalent figures were 0.58 (27), 1.00 (6), 1.30 (67) and 1.29 (20). For stroke the figures were 0.75 (56), 0.69 (7), 1.12 (97) and 1.39 (38). Observed to expected ratios for pulmonary embolism, for the ethinylestradiol 0.03mg series containing levonorgestrel at doses of 0.15 and 0.25mg were 1.14 (30) and 0.90 (32), respectively. For ischaemic heart disease the equivalent figures were 1.40 (7) and 0.67 (4). For stroke the figures were 0.46 (6) and 1.37 (26). Among the norethisterone acetate-containing oral contraceptives there was therefore a tendency for the progestogen to oppose risk of pulmonary embolism but to increase risk of arterial disease. The trend seen with progestogen dose and pulmonary embolism was not seen with superficial or DVT and has been ascribed by one of the principal investigators to chance.[12] Moreover, as described above, adverse reaction reporting is subject to a number of potential biases.

In the first report from the RCGP study, the rate of venous thrombosis relative to formulations containing less than 3mg progestogen was 0.88 for formulations containing 3mg norethisterone acetate and 0.60 for formulations containing more than 3mg progestogen.[20] However, this trend was reversed in a further analysis which distinguished deep and superficial vein thrombosis. In contrast to the possible link it demonstrated between progestogen dose and risk of arterial disease, the RCGP study did not confirm a link between progestogen dose and risk of VTE. Few other studies have been of sufficient size to analyse effectively the effects of progestogen dose on risk of VTE. The Michigan Medicaid cohort study employed a measure of progestogen potency but found no link with risk of VTE.[79,80] Findings such as these have led to the conclusion that progestogen dose has no influence on risk of VTE.[13]

Possible effects of progestogen type on risks of VTE will be considered in section 5.

Summary

There appears to be a 4-fold increase in risk of idiopathic VTE among oral contraceptive users compared with women not taking oral contraceptives. The clinical importance of this increase can best be assessed in terms of annual rates in groups of young women at differing degrees of risk. The average incidence of VTE among users of formulations containing predominantly estrogen 0.05mg can be estimated at 82 cases per 100 000 women-years.[56,82,83] Estimates of the incidence of VTE in pregnancy vary and are affected by whether only first occurrences of VTE are considered, how long into the puerperium the period of observation is continued, and by changes in diagnostic techniques.[84] The following estimates provide examples: 59 per 100 000 women-years, from a UK general practitioner database;[15] 67 per 100 000 women-years in the North-West Thames region of the UK (personal communication; S. Robinson, 2000); 85 per 100 000 women-years in Denmark between 1984 and 1994 for pregnant women and women up to 8 weeks following delivery;[84] and 90 per 100 000 women-years in Denmark currently for pregnant women alone (Lidegaard and Edström, unpublished data). A figure of 75 per 100 000 women-years may be taken for comparative purposes as representative of nonfatal VTE in pregnancy. Earlier studies of women taking predominantly levonorgestrel- or norethisterone-containing formulations combined with less than 0.05mg estrogen suggest an incidence rate of 41 cases per 100 000 women-years.[82,83] The equivalent figure for users of desogestrel- and gestodene-containing formulations was 33 cases per 100 000 women-years.[28,85,86] Among women using combinations containing predominantly levonorgestrel or norethisterone as progestogen during the early 1990s, there was an average incidence of 18 cases per 100 000 women-years.[28,85,86] The apparent reduction in risk associated with use of low estrogen dose formulations containing levonorgestrel or norethisterone has been noted before[32,87] and could be attributed to the progressive selection of healthier users as the duration of experience of these formulations in the population lengthens (see section 5). Among women not taking oral contraceptives, the average incidence is not more than 11 cases per 100 000 women-years[15] (fig. 4).

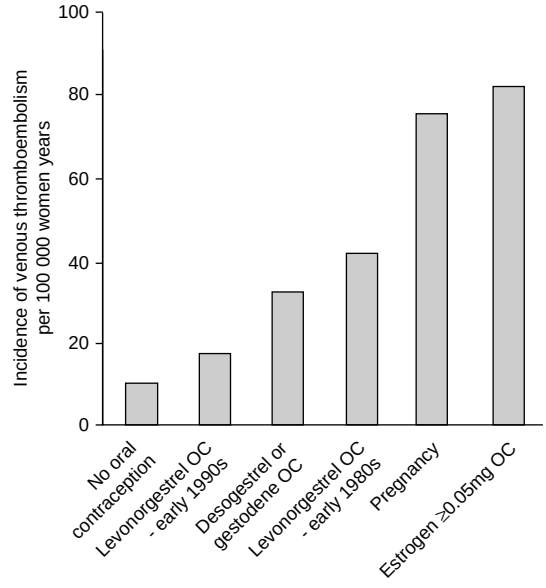

Fig. 4. Incidence of venous thromboembolism in young women during pregnancy and according to type of oral contraceptive (for references see section 2.1.3).

Candidate mechanisms for increased risk of VTE in oral contraceptive users will be considered in detail in subsequent sections. These include adverse changes in components of the haemostatic system, impairment of vessel tone leading to increased tendency to stasis, the induction of proliferative lesions in the vascular endothelium, increased homocysteine levels and an inflammatory reaction associated with an immune response to ethinylestradiol.

2.2 Myocardial Infarction (MI)

MI is the most dramatic and clearly-defined manifestation of ischaemic heart disease and so has been a major clinical end-point in studies of occlusive vascular disease in oral contraceptive users.

2.2.1 Epidemiology of MI in Young Women

According to WHO statistics for developed countries in the period 1992 to 1996,[1] the estimated rate of MI among women who were non-smokers and not taking oral contraceptives in the age ranges 20 to 24, 30 to 34 and 40 to 44 years were 0.14, 1.70 and 21.28 per million per year. Equivalent figures for death from MI were 0.04, 0.51 and 6.38. As described previously, the reporting system in Denmark makes available comprehensive national hospital admission and mortality records; incidence of and death from MI in 5-year age bands in women in Denmark between the ages 15 and 44 years from 1980 to 1993 are illustrated in figure 5. The estimated rate of MI among women (including cigarette smokers and oral contraceptive users) in the age ranges 20 to 24, 30 to 34 and 40 to 44 years were 5, 31 and 219 per million per year. Equivalent figures for death from MI were 0.8, 9.8 and 55.3. It is important to note that young women using any form of contraception tend to have a lower incidence of coronary heart disease than women of comparable age in the population as a whole.[20,22,88] This complicates any attempt to arrive at reliable reference figures. This phenomenon was particularly noticeable in the Oxford/FPA Study.[62] After more than 200 000 women-years of follow-up, the incidence of ischaemic heart disease was only 1 in 30 000 for those aged 25 to 34 years, rising to 22 in 30 000 in women aged 45 to 49 years. Mortality from ischaemic heart disease in this cohort was about one half that expected from national statistics.

2.2.2 Risk Factors for MI in Young Women

Oliver,[89] noting a 50% increase in mortality from ischaemic heart disease between 1960 and 1970 among women aged 35 to 44 years, undertook one of the first detailed evaluations of risk factor prevalence in a case series of young women with ischaemic heart disease. 150 cases, seen between 1953 and 1970, who had electrocardiographic evidence of disease were considered. This study included cases of both angina pectoris (65 cases) and MI (85 cases). 74% of cases had one or more of the risk factors: hypercholesterolaemia, hypertension or

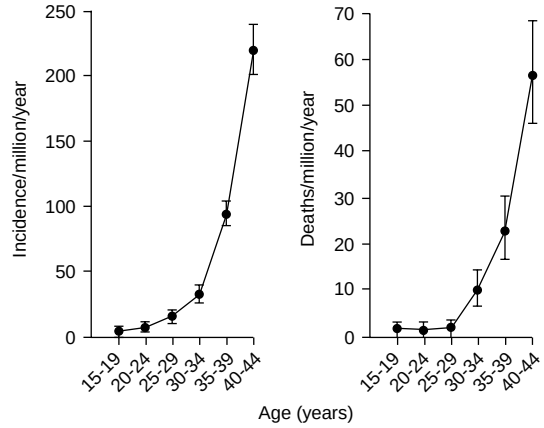

Fig. 5. Morbidity (n = 937) and mortality (n = 233) for myocardial infarction in young women in Denmark from 1980 to 1993 (rate point estimates and 95% confidence intervals are shown) [reproduced from Lidegaard,[29] with permission].

cigarette smoking. 38% had one or more of the risk factors: premature menopause, obesity, diabetes mellitus or oral contraceptive use. 11% had no predisposing risk factors. Continuing hypertension was the most important factor dictating poor prognosis.

Much detailed information on risk factors for MI in young women has come from studies in oral contraceptive users, in which risk factors in cases of MI in young women not exposed to oral contraceptives were evaluated.[21,88,90-100] From these studies the following risk factors could be distinguished: cigarettes smoking, hypertension, diabetes mellitus, hyperlipidaemia, obesity, pre-eclamptic toxaemia and family history of early MI. Other factors noted included high parity, low alcohol intake, non-O blood group, low income, low educational attainment, Black race and gallbladder disease.

Independent of oral contraceptive use, cigarette smoking was clearly identified as the most prominent risk factor for MI in young women. Relative risk estimates of 4.3[21] or 6.6[88] were reported in women smoking 15 or more cigarettes per day, and 11.3[90] or 13.1[98] in women smoking 25 or more per day. In the largest case-control analysis of the effects of cigarette smoking in women yet undertaken (555 cases and 1864 controls), Rosenberg

and colleagues[101] found that the relative risk of MI increased to 1.4, 2.4, 5.0 and 7.0, respectively, with smoking 1 to 14, 15 to 24, 25 to 34 and 35 or more cigarettes per day. Eighty percent of cases were current cigarette smokers, compared with 54% of the controls. The trend of increasing risk was apparent in all age ranges (25 to 39, 40 to 44 and 45 to 49 years), and was augmented in the youngest age range, the relative risk among those smoking 25 to 34 or 35 or more cigarettes per day being 10 and 13, respectively. The relative risk of MI with increasing cigarette smoking was analysed in (i) both oral contraceptive users and non-users (see section 2.2.3), (ii) increasing ranges of total cholesterol, (iii) according to history of hypertension, angina pectoris or diabetes mellitus, and (iv) according to menopausal status, obesity, high density lipoprotein (HDL) cholesterol, blood group, family history or personality score status. Within each risk category, cigarette smoking increased the risk of MI. However, between categories (e.g. current use-never use of oral contraceptives, history-no history of hypertension, low-high HDL cholesterol) potentiation of the increasing risk with cigarette smoking was only apparent in the categories: oral contraceptive use and elevated total cholesterol level.[101])

The effect of cigarette smoking was also apparent in a more recent large case control analysis (second only in size to that of Rosenberg et al.[101]) by Dunn and colleagues,[102] using data from the MICA study. Odds ratios relative to nonsmokers with no clinical risk factors (hypertension, hyperlipidaemia, angina pectoris or diabetes mellitus) were 2.5 (95% CI 1.1 to 5.9) for women smoking 1 to 19 cigarettes per day and 14.5 (7.2 to 29.3) for women smoking 20 or more cigarettes per day. For women with 3 or more clinical risk factors, nonsmokers had an odds ratio for MI of 6.7 (3.5 to 13.1), compared with 35.8 (17.9 to 71.8) for women smoking 1 to 19 cigarettes per day and 66.8 (34.7 to 129) for women smoking 20 or more cigarettes per day.

2.2.3 MI in Oral Contraceptive Users

A link between oral contraceptive use and MI in young women was first suggested by case reports of coronary thrombosis in oral contraceptive users during the 1960s.[103-108] Case-control and cohort studies and analyses of mortality statistics followed, and results from these have continued to appear over the last 30 years.

Case-Control Studies

Three studies in the UK, stimulated by a series of case-reports of thrombotic events in oral contraceptive users during the 1960s, provide the earliest epidemiological information on risk of MI in oral contraceptive use. In the Royal College of General Practitioners' analysis of records from 29 UK general practitioners from 1961 and 1966, there were 7 cases of MI among women aged 15 to 49.[38] However, among these records no case of MI occurred in an oral contraceptive user. In their report to the UK Committee on Safety of Drugs, Inman and Vessey[45] compared 194 cases of fatal coronary thrombosis, specified in UK death certificates for 1966 for all women aged 20 to 44 years, with 998 controls. The study was limited to non-pregnant married women and each case was examined to determine whether there was any predisposing condition or illness that might have accounted for their death. 83% of cases were confirmed by autopsy. There was an odds ratio of 1.7 (95% CI 1.0 to 3.0) for oral contraceptive use among the 84 cases without conditions that would have predisposed towards MI. The odds ratio was 0.4 (0.2 to 0.9) for the 110 cases with predisposing factors, indicating that among women with predisposing factors oral contraceptive use was less likely in those who would subsequently die of coronary thrombosis. The report of the U.K. Medical Research Council's Statistical Research Unit by Vessey and Doll,[40] analysed records from 19 UK hospitals between 1964 and 1966, on women aged 16 to 40 years, but included only 13 cases of nonfatal coronary thrombosis, none of whom were taking oral contraceptives. An extension of this study to include records from 1967 increased the number of cases of coronary thrombosis from 13 to 17, but even then only 2 cases were using oral contraceptives.[33]

These initially equivocal findings[33,38,40,45] and reports from the numerous case-control studies

that have followed them[21,48,49,78,90-100,109-121] are summarised in table IV. Several of these studies have published on more than one occasion, with the inclusion of additional cases and controls.[48,49,78,90,93,95,98,99,110,112-114,118,120] In all, there have been 22 distinct case-control studies regarding MI in oral contraceptive users (it should be noted that there has been a tendency in more recent studies to consider only those who have survived a MI, which has the advantage of enabling the maximum information to be obtained from each case, but leaves open questions concerning case fatality rates).

Among the studies listed in table IV, that of Jick et al.[93,113] had an 85% attrition rate from the original sampling frame and returned an odds ratio for MI that was almost 4 times higher than the highest estimate from all other studies. In the analysis of death certificates from 5 US metropolitan centres by Kreuger et al.,[96] there were uncertainties over assignment of cause of death, particularly among the 51 cases who were Black. When the analysis was restricted to White cases with no predisposing conditions and a definite diagnosis of MI (22 cases including 10 oral contraceptive users) an odds ratio of 4.2 (95% CI 1.4 to 12.0) was found. Women taking postmenopausal estrogen replacement therapy were included in the study of Talbot et al.[115]

The analysis by Thorogood et al.[92] of UK death certificates from 1986 to 1988 which found an odds ratio of 1.9 (95% CI 0.7 to 4.9) among those with no predisposing factors, is of particular interest. This study was very similar in design to an earlier analysis relating to 1966,[45] 1973[78,112] and 1978[91] UK death certificates. Odds ratios were 1.7 (95% CI 1.0 to 3.0), 2.2 (1.3 to 3.8) and 2.2 (1.2 to 4.4), respectively. The analyses of 1966 and 1978 death certificates included only those individuals with no predisposing conditions. The analysis of 1973 death certificates did not undertake this subanalysis. If it had, an odds ratio in excess of 2.2 would have been expected (on the basis of the average differential observed in other such subanalysis, an odds ratio of around 3.0 is possible). These data suggest a declining risk of death from MI in oral contraceptive users from 1973, to the latter part of the 1980s among oral contraceptive users with no predisposing conditions.

Excluding the study of Jick et al.,[93,113] mentioned above, in which an odds ratio of 15 was found, and taking the overall estimate in the most inclusive study publication, the average odds ratio for MI among oral contraceptive users, weighted by the number of cases studied, was 2.1. It must be recognised, however, that the estimates on which this average is based were derived from study groups which included oral contraceptive users with risk factors for MI, primarily cigarette smoking. Statistical adjustments were made for these factors in a number of the analyses, but it is doubtful whether these would have entirely eliminated the effect of the risk factor.[14]

Subanalyses of women with no known predisposing conditions (with the exception, in most cases, of cigarette smoking) were described in a total of 9 studies.[45,91-93,95-97,100,113,114] In all but one (the only one in which cigarette smokers were excluded)[100] the odds ratio was higher in the analysis of women without predisposing conditions compared with that for the overall analysis. An elevated odds ratio in women without predisposing conditions is consistent with women with predisposing conditions not being prescribed oral contraceptives. This follows since inclusion of women with predisposing conditions would lead to an over-representation of women at increased risk of MI in the non-user control group. This would, in turn, lead to a bias towards increased risk of MI in the control group, and a reduction in the relative risk associated with oral contraceptive use.

Odds ratios for MI among women with no known predisposing conditions ranged between 1.4 and 4.6 (again excluding the study of Jick et al,[93,113] referred to above, in which an odds ratio of 15 was found). The lower limit of the confidence interval for 6 of the estimates was greater than 1.0. As mentioned, cigarette smokers were included in all but the WHO Collaborative Study analyses, and it is unlikely that statistical adjustment eliminated all the effects of smoking from the risk estimate.[14] This may be particularly so for cigarette smoking,

Table IV. Case-control studies of myocardial infarction in oral contraceptive (OC) users

Date and source of cases	No. of cases	OC user cases	Odds ratio (95% CI)	Reference
1961-66 UK GP records	7 MI	0		RCGP[38]
1964-67 UK hospital records	17 nonfatal MI	2		Vessey & Doll[33,40]
1964-68 US hospital records	63 nonfatal MI	8	1.8 (0.8-4.4)	Arthes & Masi[109]
1964-76 US nurses	156 nonfatal MI	23	1.6 (1.0-2.6)	Rosenberg et al.[99]
	17 nonsmokers, NPC	5	2.8 (1.0-7.8)	
1966 UK death certificates	194 fatal MI	23	1.0 (0.6-1.3)	Inman & Vessey[45]
	84 NPC	18	1.7 (1.0-3.0)	
1968-72 UK hospital records	72 nonfatal MI	20	4.2 (2.1-8.4)	Mann et al.[90,110]
?1970s US hospital records	34 nonfatal MI	4	1.9 (0.5-1.6)	Rosenberg et al.[111]
1970-73 US hospital records	28 nonfatal MI, PC	?	1.3 (0.4-4.2)	Stolley et al. McGuire et al.[48,49]
1973 UK death certificates	199 fatal MI	41	2.2 (1.3-3.8)	Mann et al.[78,112]
1974-75 US death certificates	163 fatal MI	20	1.2 (0.6-2.3)	Kreuger et al.[96]
	51 NPC	7	1.4 (0.5-3.8)	
1975 US hospital records	30 nonfatal MI, NPC	23	15.0	Jick et al.[93,113]
1976-78 US hospital records	234 nonfatal MI	29	4.0 (2.5-6.3)	Shapiro et al.[95]
	90 NPC	?	5.6	
	38 nonsmokers	4	4.5 (1.4-14.1)	
1976-79 US hospital records	556 nonfatal MI	41	3.5 (2.2-5.5)	Slone et al.[114]
	NPC (numbers not given)		3.9 (2.0-7.6)	
1977-78 US death certificates	42 sudden cardiac death	3 ≤50y	2.8 (0.4-19.0)	Talbot et al.[115]
1978 UK death certificates	139 fatal MI	24	1.4 (0.8-2.4)	Adam et al.[91]
	89 NPC	22	2.2 (1.2-4.4)	
1983-85 Italian hospital records	168 nonfatal MI	6	2.1 (0.7-7.1)	La Vecchia et al.[98]
1983-92 Italian hospital records	251 nonfatal MI	7	2.0 (0.7-6.7)	D'Avanzano et al.[118]
1983-86 Hungarian hospital records	59 nonfatal MI	0		Ananjevic-Pandey et al.[116]
1985-89 US hospital records	910 nonfatal MI	6	1.1 (0.4-3.1)	Rosenberg et al.[117]
1986-88 UK death certificates	161 fatal MI	37 [a]	1.1 (0.6-2.4)	Thorogood et al.[92]
	106 NPC	26	1.9 (0.7-4.9)	
1989-95 European hospital records	198 nonfatal MI	62 [a]	5.0 (2.5-9.9)	WHO Collaborative Study[100]
	26 NPC (including smoking)	10	3.1 (1.1-9.0)	
1989-95 Developing country hospital records	170 nonfatal MI	39[a]	4.8 (2.5-9.1)	
	48 NPC (including smoking)	11	4.0 (1.6-10.1)	
1991-94 US population sample	130 fatal or nonfatal MI	10[a]	1.7 (0.5-6.1)	Sidney et al.[99]
1991-95 US population sample	271 fatal or nonfatal MI	87[a]	0.9 (0.4-2.2)	Sidney et al.[120]
1993-95 European hospital records	147 nonfatal MI	23[ab]	3.1 (1.5-6.3)	Lewis et al.[119]
1993-96 European hospital records	182 nonfatal MI	57	2.4 (1.4-3.9)	Lewis et al.[122,123]
1993-95 UK hospital and mortality records	448 fatal or nonfatal MI	59[a]	1.4 (0.8-2.5)	Dunn et al.[121]

a Odds ratios adjusted for potential confounding variables (e.g. age, diabetes mellitus, cigarette smoking, hypertension, hypercholesterolaemia).

b Users of combined oral contraceptives containing levonorgestrel or norethisterone.

CI = confidence interval; **MI** = myocardial infarction; **NPC** = no predisposing conditions – generally previous cardiovascular disease including hypertension and preeclampsia, diabetes mellitus and hyperlipidaemia; **PC** = predisposing conditions.

in view of the potentiation of effect when smoking and oral contraceptive use are present together (see below). Several of the larger studies undertook subanalyses relating to women who did not smoke and found the following odds ratios: 2.8 (95% CI 1.0 to 7.8);[111] 4.5 (1.4 to 14.1);[95] 1.3 (0.6 to 2.8) – unadjusted for age;[101] 0.9 (0.3 to 2.7);[21] 3.1 (1.1 to 9.0).[100] As would be expected from the marked exponential increase in risk with age, the majority of cases in these analyses were women over 35 years of age. However, these analyses do suggest that increased risk of MI in oral contraceptive users cannot be entirely attributed to cigarette smoking. In the WHO Collaborative Study, the odds ratio of

3.1 (1.1 to 9.0) for oral contraceptive use and MI in nonsmokers was considerably diminished and became nonsignificant when those who had not had a blood pressure (BP) check were excluded. It is possible that in other studies that found increased odds ratios in nonsmokers, exclusion of women who had not had a BP check would have similarly eliminated the increased risk of MI.

Cohort Studies

Prospective analyses of risk of MI in cohort studies are summarised in table V.[18,22-24,27,58,60,62,63,124,125] Of these, the Walnut Creek Contraceptive Drug Study[24] and the Puget Sound Health Cooperative Study[27,63,125] are of limited value, since the former included only 2 and the latter only 1 case of MI among ever users or current users of oral contraceptives. The Nurses' Health Study found a relative risk of 2.5 for coronary heart disease in current users,[23] and the 20 year follow-up of the Oxford/FPA Study found a relative risk of 3.3 for fatal ischaemic heart disease in ever-users[62] (in the latter, the majority of fatal cases of ischaemic heart disease were in women who had stopped taking oral contraceptives because of the previous onset of health problems while taking oral contraceptives). The RCGP Study analysis of first events of cardiovascular disease, published in 1983, found a relative risk of 2.0 in 24 cases of fatal or nonfatal MI among current users.[124] This relative risk was appreciably lower than the relative risk of 6.4 for exclusively fatal ischaemic heart disease found in a previous analysis.[58] The authors ascribed the greater risk of cardiovascular mortality in oral contraceptive users to a greater case fatality rate in oral contraceptive users who smoked. In general, the cohort studies indicate a relative risk of 2 to 3 for MI in current oral contraceptive users, which accords with the findings from the case-control studies.

Joint Effects with Other Risk Factors

The first suggestion that the effects of oral contraceptive use and other risk factors might combine to greatly augment risks of MI came from the case series described by Oliver in 1970.[108] Among women aged 41 years or less admitted to a coronary care unit between 1965 to 1969, 50% were oral contraceptive users, an appreciably higher proportion than would have been expected from the prevalence of oral contraceptive use in the community. However, 9 of the 11 oral contraceptive users had other risk factors for MI, including hypertension, hyperlipidaemia and cigarette smoking, suggesting aggravation of an existing predisposition. A similar conclusion was reached in a subsequent report on cases seen between 1970 and 1972.[126]

The possibility that risks of MI might be potentiated by the joint effects of oral contraceptive use and other risk factors led to widespread selective prescribing, whereby women with known risk factors were discouraged from taking oral contraceptives.[91] This will have influenced findings in many studies. However, results presented in the first report from the Nurses' Health Study on MI in oral contraceptive users[97] are less likely to have been thus affected since this was a retrospective case-control analysis of events occurring between 1964 and 1976 during which less awareness of possible risks of MI and oral contraceptive use might have been expected. Compared with women who had never used oral contraceptives and who had no predisposing factors for MI, oral contraceptive users with no other predisposing factors had an odds ratio of 2.8, cigarette smokers with no other factors had an odds ratio of 5.0, and women with hypertension with no other risk factors an odds ratio of 7.6. Women with no other predisposing factors except oral contraceptive use and cigarette smoking had an odds ratio of 5.6, and those who were hypertensive smokers an odds ratio of 8.9. For women with all 3 risk factors the odds ratio was 170.

Such effects were also apparent in the first study of Mann et al.[90] Joint effects among the risk factors, hypercholesterolaemia, treated hypertension, cigarette smoking, treated diabetes mellitus, current oral contraceptive use and obesity were evaluated by considering risk of nonfatal acute MI in individuals with 1, 2 or 3 risk factors. Relative to those with no risk factors, risk increased from 4.2 to 10.5 to 78.4, respectively. The cumulative magnitude of such combined effects was particularly

Table V. Cohort follow-up studies of myocardial infarction in oral contraceptive (OC) users

Date	OC Status	Women-year follow-up	No. of cases	Relative risk (95% CI)	Reference
UK Royal College of General Practitioners' Study					
1968-72	Never users	42 306	1 MI		
	Current users	34 875	5 MI	5.2	20
1968-76	Never users	2 fatal MI			57
	Current users	6 fatal MI	3.2		
1968-79	Never users	138 630	3 fatal IHD		58
	Current users	98 997	10 fatal IHD	6.4 (1.7-23.5)	
1968-79	Never users	129 593	20 MI[a]		124
	Current users	98 551	24 MI[a]	2.0 (1.1-3.5)	
1968-87	Current users: nested case-control analysis		91 MI	1.8 (0.9-3.6)	21
			47 nonsmokers	0.9 (0.3-2.7)	
Oxford / Family Planning Association Study					
1968-74	Never users	34 035	0		
	Current users	21 794	2 MI		22
1968-80	Never users	62 532	1 fatal IHD		
	Ever users	79 678	8 fatal IHD	-	60, 61
1968-87	Never users	118 671	3 fatal IHD		
	Ever users	152 597	15 fatal IHD	3.3 (0.9-17.9)	62
Walnut Creek Contraceptive Drug Study					
1968-77	Never users	37 913	15 MI		
	Ever users	20 134	2 MI	1.1 (0.4-11.5)	24
1969-76	Current users: nested case-control analysis		26 MI	0.8 (0.2-2.8)	94
Puget Sound Health Cooperative Study					
1977-82	Non-users	245 567	1 MI		
	Current users	54 971	0	-	27, 63, 125
Nurses Health Study					
1976-84	Never users	484 096	339 CHD	2.5 (1.3-4.9)	23
	Current users	22 376	10 CHD		

a Fatal or nonfatal MI as a first episode of any vascular disease.

CHD = coronary heart disease; **CI** = confidence interval; **IHD** = ischaemic heart disease; **MI** = myocardial infarction.

high in the WHO Collaborative Study.[100] 82% of oral contraceptive users who experienced a MI had at least one other risk factor (hypertension, rheumatic heart disease, diabetes mellitus, abnormal blood lipids, hypertension in pregnancy or cigarette smoking) and this was associated with more than a 10-fold increase in risk of MI compared with that seen in oral contraceptive users with no risk factors (odds ratios of 37.3 and 3.1, respectively).

Whilst the effect of factors such as hypertension, diabetes mellitus and hyperlipidaemia would have become less important with more selective prescribing, possible interactions with cigarette smoking, which has increased in young women, remains a concern. An excess of smokers (>80%) among cases who were oral contraceptive users was apparent in the great majority of studies[14,21,90,91,93,100,101,110,117,127-129] [among studies published prior to 1996, the only exception has been a prospective analysis from the RCGP study, in which ever-use of oral contraceptives was associated with a relative risk for circulatory disease mortality of 3.2 (95% CI 1.1 to 9.0) for nonsmokers and only 5.1 (2.4 to 10.9) for smokers[58]].

The largest analysis of this issue (555 cases, 1864 controls) was by Rosenberg et al.,[101] based

on data first presented by Shapiro et al.[95,114] Among women who had never used oral contraceptives, relative to nonsmokers women who smoked 1 to 24 cigarettes per day had an odds ratios for MI of 2.0 (95% CI 1.4 to 2.8), and women who smoked 25 or more cigarettes per day had an odds ratio of 4.8 (3.5 to 6.6). Among women currently using oral contraceptives, the equivalent figures were 3.1 (0.4 to 22) and 23 (6.6 to 82), respectively (fig. 6). These estimates were made with regard to different reference groups and are therefore not strictly comparable. However, the odds ratio in oral contraceptive users who smoked heavily was appreciably greater than would be expected from the odds ratios associated with oral contraceptive use or cigarette smoking alone.

The combined effect of heavy smoking and oral contraceptive use was also apparent in the nested case-control analysis of the RCGP cohort published by Croft and Hannaford.[21] Odds ratios for first acute MI relative to nonsmokers not using oral contraceptives were 2.0 (95% CI 1.0 to 3.9) in non-users smoking fewer than 15 cigarettes per day and 3.3 (1.6 to 6.7) in non-users smoking 15 or more cigarettes per day.[21] In oral contraceptive users, the equivalent figures were 3.5 (1.3 to 9.5) and 20.8 (5.2 to 83.1) [fig. 6].

The WHO Collaborative Study also revealed a marked increase in risk of MI in oral contraceptive users who smoked 10 or more cigarettes per day [relative risks in current oral contraceptive users in European centres: non smokers 4.0 (95% CI 1.5 to 10.4); less than 10 cigarettes per day 5.0 (0.8 to 32.4); 10 or more cigarettes per day 87.0 (29 to 254)].[100] This was also the case for the Transnational Study [relative risks in current oral contraceptive users: non smokers 1.6 (0.6 to 4.5); less than 10 cigarettes per day 1.3 (0.1 to 12.1); 10 or more cigarettes per day 43.7 (9 to 202)[14]].

However, at the time of writing (August 2000), the two most recently published studies of MI in oral contraceptive users are in striking contrast to earlier reports, in that they appear to have found evidence of no joint effect between oral contraceptive use and cigarette smoking.[102,120,121] In a

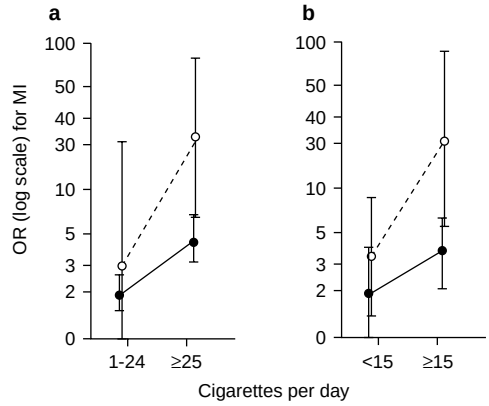

Fig. 6. Joint effects of oral contraceptive use and cigarette smoking in increased risk of myocardial infarction (MI). Open circles, broken line: oral contraceptive users. Closed circles, continuous line: women not taking oral contraceptives.
(**a**) For oral contraceptive users odds ratios (ORs) were estimated relative to nonsmokers using oral contraceptives and for non-users relative to nonsmokers not using oral contraceptives.[101] (**b**) All odds ratios were estimated relative to nonsmokers not using oral contraceptives.[21]

population-based study by Sidney and colleagues[120] of women in California and Washington State, US (271 cases and 993 controls), there was no increase in risk of MI with oral contraceptive use, regardless of whether women were smokers, overweight, 40 years of age or more or had hypertension, hypercholesterolaemia or diabetes mellitus. Compared with women not taking oral contraceptives and who did not smoke, there was, however, a relative risk of MI of 1.9 (95% CI 0.6 to 6.3) in current oral contraceptive users who did not smoke and 5.9 (0.9 to 40.6) in those who did smoke,[14] so some joint effect is not excluded (effects of light, heavy or moderate cigarette smoking were not distinguished in this analysis). However, in the MICA study, based on hospital and mortality records in the UK (448 cases, 1728 controls), there was unequivocal evidence for a lack of joint effect between cigarette smoking and oral contraceptive use.[102,121] Compared with women not taking oral contraceptives, among nonsmokers the odds ratio for MI was 1.0 (0.4 to 2.3) for oral contraceptive use; among women smoking 1 to 19 cigarettes per

day the odds ratio was 1.8 (0.8 to 4.0) for oral contraceptive use; and among women smoking 20 or more cigarettes per day there was an odds ratio of 1.0 (0.5 to 1.9) for oral contraceptive use.[102] This analysis was second-only in size to that of Rosenberg and colleagues,[101] so the disappearance of any joint effect between oral contraceptive use and cigarette smoking could reflect a genuine change in risk patterns. One difference between the two reports is that in the analysis by Rosenberg et al.,[101] odds ratios were presented in terms of the risks of cigarette smoking according to oral contraceptive use (i.e. odds ratios for MI among women smoking 1 to 24 and 25 or more cigarettes per day and using oral contraceptives were estimated relative to nonsmoking current oral contraceptive users, and odds ratios for MI among women smoking 1 to 24 and 25 or more cigarettes per day who had never used oral contraceptives were estimated relative to nonsmoking never users of oral contraceptives). In the report from the MICA study,[102] odds ratios were estimated in terms of the risks of oral contraceptive use according to cigarette smoking. Crude odds ratios comparable with those presented by Rosenberg et al.[101] may be estimated for the MICA Study, using the numbers of cases and controls given.[102] Among women not taking oral contraceptives, the odds ratio for MI for women smoking 20 or more cigarettes per day compared with nonsmokers was 16.6 (11.5 to 24.1), whereas among oral contraceptive users the equivalent figure was 17.2 (6.0 to 49.9), thus providing further support for a lack of effect of oral contraceptive use on risks of MI associated with cigarette smoking in this study. The major difference between these two studies lies in the differing compositions of the oral contraceptive formulations being used. In the study of Rosenberg,[101] the majority of users would have been taking formulations containing 0.05mg or more of estrogen combined with a norethisterone-type or levonorgestrel progestogen, whereas in the MICA study the great majority were taking formulations containing less than 0.05mg estrogen combined with desogestrel, gestodene or levonorgestrel as progestogen.

As described in the following section on variation in risk with oral contraceptive formulation, evidence for an association between estrogen dose and risk of MI is equivocal. In the WHO Collaborative Study, a joint effect of oral contraceptive use and cigarette smoking was still apparent despite a substantial proportion of users taking formulations containing less than 0.05mg estrogen.[100] However, as the MICA study authors noted,[102] there were still relatively large numbers of cases and controls (31 cases and 43 controls) taking higher dose oral contraceptives in the WHO Collaborative Study, whereas in the MICA study such numbers were very small (1 case and 2 controls), so an influence of estrogen dose was not excluded in the WHO study. Moreover, the possibility of such an effect was supported by data from the Transnational Study which returned an odds ratio of 19.5 (95% CI 2.1 to 180) for MI among cigarette smokers using oral contraceptives containing 0.05mg or more of estrogen compared with nonsmokers not taking oral contraceptives.[122] The equivalent figure for cigarette smokers taking formulations containing less than 0.05mg estrogen and predominantly levonorgestrel or norethisterone progestogens was 9.5 (2.9 to 31) and for cigarette smokers not taking oral contraceptives 6.7 (3.9 to 11.4). However, varying simultaneously with the proportion of users of high estrogen dose formulations between the WHO Collaborative and MICA studies were the proportions of users of low estrogen dose formulations containing the progestogens desogestrel or gestodene (3 cases and 5 controls for the WHO Collaborative Study, and 21 cases and 61 controls in the MICA study), so there was also the possibility of a reduction in the joint effects of oral contraceptive use and cigarette smoking by desogestrel- or gestodene-containing formulations. Again, the possibility of such an effect is supported by data from the Transnational Study.[122] Differential effects of progestogens on the joint effects of oral contraceptive use and cigarette smoking in risk of MI are examined in more detail in section 5.6.1.

Evaluation of the joint effects of age and oral contraceptive use in risk of MI is difficult because

of the exponential increase in risk of MI with age. Inevitably, more cases will be found in the upper range of the age distribution. Given the relatively small number of cases involved, statistical significance may only be found among older women and may give the impression that such risks are only important in older women. However, this does not exclude the possibility that studies may simply have lacked sufficient power to detect risks in younger women as significant. Age stratified analyses of MI risk that have found increased relative risks or odds ratios among older compared with younger women have commanded considerable attention,[57,78] but such differentials have not always been seen.[100,112]

The joint effects of oral contraceptive use and hypertension on risk of MI were explored in some detail in the WHO Collaborative Study.[100] Among Europeans, women not taking oral contraceptives who had a history of hypertension had an odds ratio for MI of 5.4 (95% CI 2.4 to 12.4) relative to non-users with no history. Oral contraceptive users with no history of hypertension had an odds ratio of 3.9 (1.9 to 7.9), but those with a history of hypertension had an odds ratio of 68.1 (6.2 to 751) relative to non-users. By any model of joint effect, the magnitude of this odds ratio strongly suggests that there is synergy between oral contraceptive use and high BP in augmenting the risk of MI; in other words, the joint effect of the two risk factors together is appreciably greater than the effect of the two separately. In the WHO Collaborative Study account was also taken of whether a woman had recently had her BP checked. Odds ratios for MI were generally about 3 to 4-fold higher in oral contraceptive users who had not had their BP checked. Thus, among all Europeans, the odds ratio was 9.5 (3.7 to 24.1) among those who had not had their BP checked and 2.6 (1.2 to 5.9) for those who had. The meaning that can be ascribed to an oral contraceptive user having had a BP check is uncertain. For example, she may have been monitored because of better access to healthcare, greater awareness on her own part of the need for such measurements, or because of a perceived increase in risk of vascular disease due to her age or the results of a previous BP check. It is, nevertheless, possible that BP checking has an effect in diminishing the number of oral contraceptive users with previously undiagnosed hypertension and, in so doing, diminishes the risk of MI associated with oral contraceptive use. The WHO Collaborative Study was of sufficient size to enable stratified analyses according to oral contraceptive use, cigarette smoking and BP checking, thus enabling elucidation of the effects of oral contraceptive use and smoking together, in the absence of raised BP, and the effects of oral contraceptive use and undetected raised BP in the absence of cigarette smoking (fig. 7).

Overall, the weight of evidence indicates that the joint effects on risk of MI of oral contraceptive use and cigarette smoking, high BP and, possibly, age are greater than would be expected from the effects of these factors alone. The number of younger oral contraceptive users who experience a MI in the absence of other risk factors will be extremely small. Nevertheless, a substantial proportion of oral contraceptive users do have other risk factors, in particular cigarette smoking.[130,131] The two most recently published case-control studies do, however, raise questions about other influences on the joint effect of oral contraceptive use and cigarette smoking in increasing risk of MI,[120,121] in particular, the possible influences of oral contraceptive composition, both with respect to the estrogen dose and type of progestogen.

Effects of Duration of Current Oral Contraceptive Use

There have been some indications of an increased risk of MI in oral contraceptive users with increasing duration of use in case-control studies[78,91,114] but trends were generally non-significant.[91,114] Other studies have found no such association.[90,100] This has also been the case with cohort studies. For example, in the RCGP study an early indication of increased risk with increasing duration of use[57] was not found in subsequent analyses.[58] Therefore, the weight of evidence argues against an increase in risk with increasing duration of use. It should be kept in mind that long

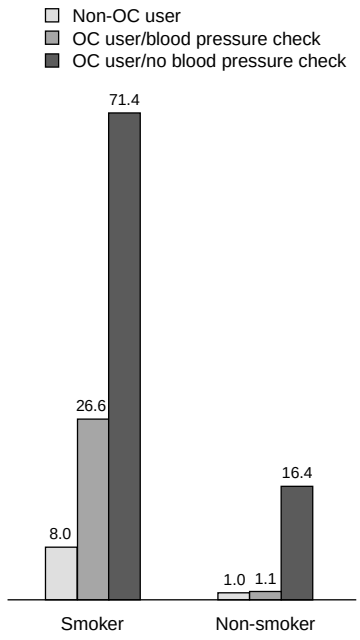

Fig. 7. Odds ratios for myocardial infarction (shown on each column) according to oral contraceptive (OC) use, cigarette smoking, and whether or not a blood pressure check had been made.[100]

term users of oral contraceptives are a self-selected group who have found this mode of contraception satisfactory. In accord with this, one of the most recent studies found a progressive, albeit non-significant decrease in risk of MI with increasing current duration of use (ORs of 1.04, 1.60 and 0.35 with <4, 5 to 9 and >10 years of use, respectively).[120]

Effects of Past Use of Oral Contraceptives

The great majority of case-control studies of MI risk in past users of oral contraceptives indicate little residual risk.[21,78,90-92,95,97,100,117,120,124,132] In two relatively small case-control studies an increased risk was found in past users of oral contraceptives, but this may relate to women who stopped using oral contraceptives because of adverse effects.[98,116] That such an effect can operate was confirmed in the RCGP and Oxford/FPA cohort studies.[68,60-62]

There have been two large studies suggesting that there may be a residual effect of past use. In the report by Slone et al.,[114] the overall odds ratio for past users relative to never users was 1.2 (95% CI 0.9 to 1.4). However, when the data in past users was analysed according to duration of past use, a significant increase in risk with increasing duration of use was apparent in the older age range. Among those aged 25 to 39 years there were non-significant odds ratio, relative to never users, of 0.8, 0.8 and 1.5 in past users who had used oral contraceptives for less than 5, 5 to 9 and 10 or more years, respectively. Among those aged 40 to 49 years the equivalent ratios were 1.0, 1.6 and 2.5, significant in the latter two categories and showing a significant trend. This link therefore related to older women using high dose formulations. The other association with past use was apparent in the 12-year follow-up analysis of all cardiovascular mortality in the Nurses' Health Study cohort (1976 to 1988) which concerned mortality in women who had never used oral contraceptives compared with ever users, the great majority of whom were past users.[133] A significant decrease in risk was found in the women who had previously been medium term users (1 to 5 years) and an increase in risk was found among those who had previously used oral contraceptives for 10 years or more. This risk in previous long term users was reduced when self-reported hypertension and hypercholesterolaemia were included in the multivariate analysis.

The weight of evidence is consistent with there being no residual effect of oral contraceptive use on risk of MI. Two of the most powerful studies found some evidence, one with regard to total cardiovascular mortality. However, in such analyses there will be marked confounding effects from the age of the participants and from accompanying changes in other risk factors such as cholesterol and BP levels. Whether it is possible to accurately distinguish any past use effects is, therefore, questionable.

Variation in Risk with Oral Contraceptive Formulation

Studies of adverse reaction reports, used on two occasions to assess differences between different formulations in their associated risks of coronary thrombosis or ischaemic heart disease, have been described in the previous section on VTE.[45,77] Variation in risk of coronary occlusion with steroid dose was not entirely consistent, although there was some suggestion of increasing risk with increasing dose of progestogen and estrogen.

With regard to case-control studies, in their report on mortality from MI, Mann and Inman[78] found no over-representation of users of high estrogen dose formulation (prior to 1970) among the cases they studied. Similarly Shapiro et al.[95] found no variation in MI risk according to estrogen content (>0.05, 0.05 or <0.05mg). Adam et al.[91] found no difference between formulations containing estrogen 0.05 or 0.03mg, both being associated with significantly increased risk. In this latter study, among women taking oral contraceptives containing estrogen 0.05mg or more the relative risk of MI was 2.3 (95% CI 1.1 to 5.0) and among those taking lower dose formulations the relative risk was 2.2 (0.8 to 5.6). Twice as many cases were taking the higher dose formulations. In the WHO Collaborative Group study, there was some evidence for a greater risk of MI among users of higher estrogen dose formulations.[100] For European study centres, odds ratios were almost identical [<0.05mg estrogen: 4.7 (2.0 to 11.0); ≥0.05mg estrogen: 4.5 (2.0 to 10.0)], whereas for developing country centres there was a higher risk among users of higher estrogen dose formulations [<0.05mg estrogen: 2.9 (1.2 o 7.0); ≥0.05mg estrogen: 7.7 (3.3 to 18.0)]. Higher odds ratios among users of higher dose formulations were seen for both European and developing country centres when the analysis was restricted to women who had not had their BP checked. Among those who had received a BP check, the risk differential was maintained for the developing country centres. In the Transnational Study, the odds ratio for MI among users of formulations containing <0.05mg estrogen and predominantly levonorgestrel or norethisterone as progestogen was 3.0 (1.5 to 5.7), whereas for users of formulations containing ≥0.05mg estrogen (which would have contained mainly norethisterone or levonorgestrel as progestogen) the odds ratio was 4.3 (1.6 to 11.7).[122] With the exception of the Transnational Study analysis, these comparisons were entirely uncontrolled for oral contraceptive progestogen content, which will have varied with estrogen dose.

The RCGP Study provided strong evidence for a positive relationship between oral contraceptive progestogen dose and risk of arterial disease.[134-136] This analysis was made possible by the widespread use of 3 formulations in the first decade of the study which all contained ethinylestradiol 0.05mg as estrogen and norethisterone acetate as progestogen, but differed in the dose of norethisterone acetate used: 1, 3 and 4mg. Standardised rates of arterial disease at each dose were 1.9, 2.8 and 3.6 per 1000 women-years, respectively. This study also included information on two formulations, both containing ethinylestradiol 0.03mg and levonorgestrel (0.15 and 0.25mg) as progestogen. Arterial disease rates at these doses were 0 and 3.0 per 1000 women-years, respectively. These differentials in arterial disease were paralleled by differentials in the incidence of hypertension. In a sub-analysis of their nested case-control evaluation of 158 cases of first acute MI in the RCGP cohort, Croft and Hannaford distinguished between never users of oral contraceptives, users of levonorgestrel-containing oral contraceptives and users of oral contraceptives containing other progestogens.[137] Compared with never users, the risk in the two oral contraceptive groups were 0.6 (95% CI 0.2 to 1.3) and 1.2 (0.8 to 1.9), respectively.

In conclusion, distinguishing risks of MI associated with different oral contraceptive formulations is difficult: estrogen and progestogen contents are often changed simultaneously and differing formulations are likely to be associated with differing prescribing habits. Moreover, several influential analyses only looked at 'total' arterial disease. What little evidence there is suggests an adverse effect of high doses of norethisterone (>2mg, which

are now no longer used) but little effect of estrogen dose. Possible effects of progestogen type on MI risk are considered in section 5.

Summary

Studies of MI in oral contraceptive users have been directed towards identifying an intrinsic effect of oral contraceptive use on risk of MI. Statistical adjustment for the influence of effect modifiers has been applied. Odds ratios in the region of 2 to 3 for MI in oral contraceptive users have been identified using this approach. However, such adjustment for effect modifiers does not necessarily eliminate their effects.[14] Moreover, the residual effects of such factors following adjustment are likely to be particularly evident when the risk factor is widely prevalent, as in the case of cigarette smoking, and when such risk factors potentiate the putative effect of oral contraceptives, as in the case of cigarette smoking, hypertension and, possibly, age. If it is indeed true, as suggested by the WHO Collaborative Study, that with screening out of women with hypertension by BP checking, and exclusion of smokers, there is no increased risk of MI among oral contraceptive users, the simplest interpretation of the data now available is that oral contraceptive use *per se* has not increased risk of MI, but has powerfully exacerbated the effects of existing arterial damage. Such damage can result from smoking, hypertension or aging. Given the ubiquity of arterial damage in economically developed societies, it is not surprising that oral contraceptive use has been so often linked with MI, but given the variability with which such damage has been accounted for in the analyses undertaken, it is equally unsurprising that findings in this respect have been inconsistent. In future studies of arterial disease among oral contraceptive users, it might be more useful to assume that oral contraceptives confer no intrinsic risk of arterial disease, but that they can profoundly modify the effects of established risk factors. The question is then: what is the magnitude of risk factor effect modification by oral contraceptives? – rather than what is the magnitude of oral contraceptive effect modification by risk factors? This is a fine distinction, but might lead to considerably greater clarity in our understanding of the realities of oral contraceptive prescribing and arterial disease. Such a perspective may be of particular value in exploring further the possible implications of the most recent studies, namely that the augmentation by oral contraceptives of the effects of risk factors for MI (especially cigarette smoking) may have been diminished by reductions in estrogen dose and by use of alternative progestogens.

2.3 Stroke and Transient Ischaemic Attack (TIA)

In the context of risks and benefits of oral contraceptives, much attention has been given to the risk of cerebrovascular diseases, including cerebral haemorrhages, subarachnoidal bleeds, cerebral thrombosis and embolism, as well as transient cerebral ischaemic attacks (TIA). It is important both from a clinical and epidemiological point of view to discriminate between haemorrhagic and thrombotic strokes, as their causes, treatments and consequences differ.

2.3.1 Incidence of Stroke and TIA in Young Women

According to WHO statistics for developed countries in the period 1992 to 1996,[1] the estimated rate of ischaemic stroke among women who were nonsmokers and not taking oral contraceptives in the age ranges 20 to 24, 30 to 34 and 40 to 44 years were 6.0, 9.8 and 16.1 per million per year. Equivalent figures for death from ischaemic stroke were 1.5, 2.5 and 4.0. Incidence of and death from cerebral thromboembolic attack in 5-year age bands in women between the ages 15 and 44 years between 1980 and 1993, according to the Danish record system, are illustrated in figure 8.[29] The estimated rates for all women in the age ranges 20 to 24, 30 to 34 and 40 to 44 years were 45, 142 and 324 per million per year. Equivalent figures for mortality were 0, 2.3 and 8.6. Approximately 4% of all cerebral thrombotic events among women are accounted for by cerebral thrombosis and TIA before the age of 45 years. The case fatality rate is low (2 to 5%) in young women. Nevertheless,

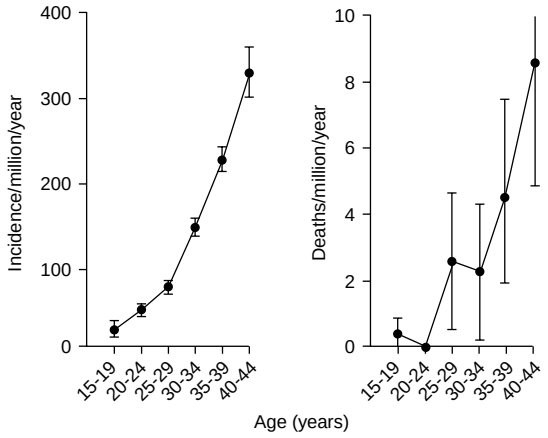

Fig. 8. Morbidity (n = 1071) and mortality (n = 48) for cerebral thromboembolic attack in young women in Denmark from 1980 to 1993 (rate point estimates and 95% confidence intervals are shown) [reproduced from Lidegaard,[29] with permission].

these diseases are important because of the serious health consequences affecting many survivors and their relatives.

The incidence rate of cerebral thrombosis in developed countries rises nearly exponentially with age,[138-140] in Denmark from 2 per 100 000 per year among 15 to 19 year old women to about 22 per 100 000 per year in women 40 to 44 years old (fig. 8).[141] This corresponds to a doubling in risk for every 5 to 6 years increase in age. The incidence rate of transient ischaemic attack also increases exponentially with increasing age, but with incidence rates of about one half of those for cerebral thrombosis.

With regard to haemorrhagic stroke, WHO statistics for developed countries in the period 1992 to 1996,[1] give an estimated rate among women who were nonsmokers and not taking oral contraceptives in the age ranges 20 to 24, 30 to 34 and 40 to 44 years of 12.7, 24.3 and 46.3 per million per year, respectively. Equivalent figures for death from haemorrhagic stroke were 3.8, 7.3 and 13.9. Among women in Denmark between the ages 15 and 44 years, the incidence rate of subarachnoidal haemorrhages increases from about 2 per 100 000 per year to 15 per 100 000 per year at 20 and 40 years, respectively (fig. 9).[141] Reports differ, however, with regard to case fatality rate in young women; according to an American study this is 16%,[142] but as high as 52% in the UK.[143] In Denmark the case-fatality rate increases from 10% among women 15 to 19 years to 22% among women 40 to 45 years old.[141]

Intracerebral haemorrhages are rarer in young women, with incidence rates about one half of those of subarachnoidal haemorrhages. The case fatality rates of intracerebral haemorrhages range from 26% in the US[142] to 82% in the UK.[143] In Denmark the case-fatality rate among women with intracerebral haemorrhages increases from 7 to 28% through reproductive ages.[141] Overall, the incidence of haemorrhages tends to be lower than the incidence of thrombotic events (including TIA), although the mortality from haemorrhagic strokes accounts for the great majority of cerebrovascular deaths in young women.

2.3.2 Risk Factors for Stroke in Young Women

The frequencies of risk factors for stroke (table VI) vary considerably in different patient samples.

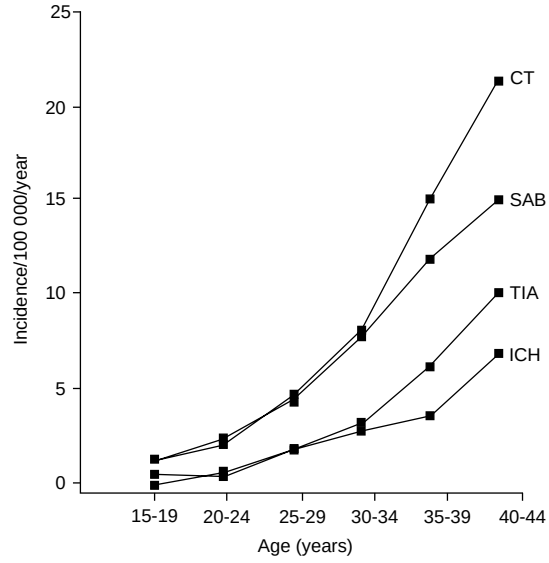

Fig. 9. Cerebral thrombosis (CT), subarachnoidal bleeds (SAB), intracerebral haemorrhages (ICH) and transitory cerebral ischaemic attacks (TIA) among young women. Incidence rates are presented according to age.[141]

Table VI. Risk factors for thrombotic strokes and transient ischaemic attack in young women. Prevalence, relative risk and aetiological fraction[144]

Risk factor	Prevalence[a] (%)	Relative risk[b] or odds ratio	Aetiological fraction[c] (%)
Smoking	40	1.5	17
Oral contraceptives	15[d]	1.8[e]	11[f]
Hypertension	4	5.4	15
Migraine	5	3.0	10
Pregnancy	3	2.0	3
Previous thrombosis	1[g]	5.3	4
Diabetes mellitus (type 1)	5	5.4	18
Medical diseases	1[h]	8.3[h]	7

a Prevalence in the normal population.
b Risk of thrombotic strokes among exposed compared with unexposed.
c Indicates the proportion of thrombotic strokes which would disappear if the specific exposure did not exist.
d Weighted according to the incidence of thrombotic strokes according to age.
e Relative risk for oral contraceptives with 30μg ethinylestradiol.
f Assuming an average relative risk among users of oral contraceptives of 1.8.
g Deep venous thrombosis, pulmonary embolism or myocardial infarction.
h Including coagulopathies, hyperlipidaemia, heart diseases, brain damage.

This may be a consequence of differences in sampling time and place and of differences in age, race, sex, diagnostic entities, variables examined and not least of how painstakingly the anamnestic, clinical and paraclinical explorations are carried out. However, in general, the most frequently published conditions associated with cerebral thrombosis and TIA in young women in developed countries are (roughly in descending order): smoking, oral contraceptive use, hypertension, migraine, diabetes mellitus, heart disease, trauma, pregnancy and puerperium, hyperlipidaemia, vasculitis, haematological diseases and coagulopathies. In many studies, young female patients with cerebral thrombosis and TIA often had as the only associated condition the use of oral contraceptives or migraine in contrast to the frequently found presence of hypertension among young male patients.[145-158]

Factor V Leiden mutation was found in a higher proportion of patients experiencing a cerebral venous thrombosis (21 *vs* 2%).[159] APC resistance was found in 3 out of 5 patients who had experienced a cerebral venous sinus thrombosis following dural puncture.[160] In a large case-control study of risk factors for ischemic stroke (202 cases, 1036 age-matched controls) carriers of the factor V Leiden mutation had an independent odds ratio for ischemic stroke of 2.56.[161] In women, the odds ratio was higher (3.95) consistent with a joint effect of factor V Leiden genotype and female hormones.

2.3.3 Stroke in Oral Contraceptive Users

The first published report linking stroke with oral contraceptive use was a case report published in 1962.[162] The epidemiological investigations undertaken to explore this relationship are described below, but two pieces of circumstantial evidence indicate the possible impact of oral contraceptives on incidence figures. Firstly, there appears to be little essential difference in stroke incidence between men and women in age group 15 to 44 years, but in those studies that do show a difference, incidence is higher in women[139,163,164] and so might be linked with the use of oral contraceptives.[165] Despite there being no valid sex-specific national incidence figures for cerebral thromboembolic attacks from the pre-oral contraceptive era, a case series from the Midland Centre for Neurosurgery and Neurology in the UK did suggest considerable impact on incidence figures.[166] Between 1954 and 1963, 25 women younger than 45 years were seen with the definite diagnosis of 'cerebral arterial insufficiency'. None had been taking oral contraceptives and they appeared at a rate of 2 to 3

per year. Between 1964 and 1973, however, 83 cases were seen, 62 of whom were using oral contraceptives.

Case-Control Studies

During the last two decades, many case-control studies have reported a significantly increased risk of thrombotic stroke among women currently using oral contraceptives. Most of these studies have been reviewed previously[6,13,167,168] and the results of all case-control studies to date are summarised in table VII. It can be seen from the table that the published relative risks have tended to decline with time. The average odds ratio, weighted by number of cases, was 10.0 for studies that considered cases occurring during the 1960s, 3.2 for studies during the 1970s and 1980s (excluding the study of Chang et al.,[175] 1986, in which ever rather than current users of oral contraceptives were considered) and 2.4 for studies during the 1990s. This impression is supported by the analysis by one of us (Lidegaard[138]) of stroke incidence over a 13-year period, and of published studies that include incidence figures. Seven recent studies have reported data on the influence of low-estrogen dose oral contraceptives on thrombotic stroke (fig. 10).[167,177,178,180-183] Four of these demonstrated a linear decrease in risk of thrombotic stroke with decreasing oral contraceptive estrogen content.[167,177,178,183] Two recent case-control studies have examined the relationship between oral contraceptive use and the rare condition of cerebral sinus thrombosis. Odds ratios of 22 (5 to 84)[184] and 13 (5 to 37)[185] were found, indicating a particularly strong association between this condition and oral contraceptive use.

Case-control studies of the risk of subarachnoidal bleeds and intracerebral haemorrhage in oral contraceptive users are listed in table VIII. The

Table VII. Case-control studies of thromboembolic stroke in oral contraceptive (OC) users

Date and source of cases	No. of cases (OC users)	Odds ratio (95% CI)	Reference
1961-66 GP records; cerebral embolism and thrombosis	3 (1)	2.5 (0.1-72)	38
1963-70 Göttingen hospital records; cerebrovascular occlusion	14 (4)	1.0 (0.3-3.4)	169
1964-66 UK hospital records; nonfatal cerebral thrombosis	19 (11)	6.1 (2.5-15.1)	33, 40
1965-67 US hospital records; idiopathic 'intracranial vascular lesion'	13 (8)	19.2 (2.5-149)	42, 43
1966 UK death certificates; fatal idiopathic thrombotic stroke	10 (5)	5.7 (1.9-17.3)	45
1969-71 US hospital records; nonfatal thrombotic stroke	140 (59)	9.5 (4.1-22.0)	170, 171
1969, 1972 multinational and US hospital records; idiopathic stroke	14 (11)	26 (7.2-91)	172
1973-77 Swedish hospital records; cerebral thromboembolism	32 (20)	4.5 (2.1-9.7)	173
1975-83 US; cerebral thromboembolism	25 (10)	3.7 (1.6-8.5)	174
1978-80 Taiwan hospital records; non-traumatic stroke	323 (55)	0.9 (0.6-1.2)[a]	175
1985-89 Danish hospital records; cerebral thromboembolism	320 (116)	3.0 (2.3-3.9)	167
1986-88 UK death certificates; fatal occlusive stroke	21 (9)	4.4 (0.8-24.4)	176
1989-93 European hospital records; ischaemic stroke	141 (52)	3.0, 1.7-5.4	177
1989-93 developing country hospital records; ischaemic stroke	556 (109)	2.9, 2.2-4.0	177
1990-93 French hospital records; nonfatal ischaemic stroke	72 (41)	3.1 (1.2-8.2)	178
1991-94 US population sample; ischaemic cerebral infarction	144 (17)	1.2 (0.5-2.56)	179
1991-95 US population sample; nonfatal ischaemic stroke	58 (6)	1.4 (0.5-3.8)	180
1991-95 US population sample; nonfatal ischaemic stroke	156 (15)	1.1 (0.5-2.2)	181
1993-95 European hospital records	220 (124)	2.9 (2.0-4.0)	182
1994-95 Danish hospital records; cerebral thromboembolism[b]	219 (22)	2.4 (1.4-4.2)	183
1994-95 Danish hospital records; cerebral thromboembolism[c]	219 (24)	1.3 (0.8-2.2)	183

a Ever users of oral contraceptives considered.

b Low estrogen dose formulations containing levonorgestrel or norgestimate.

c Low estrogen dose formulations containing desogestrel or gestodene.

CI = confidence interval.

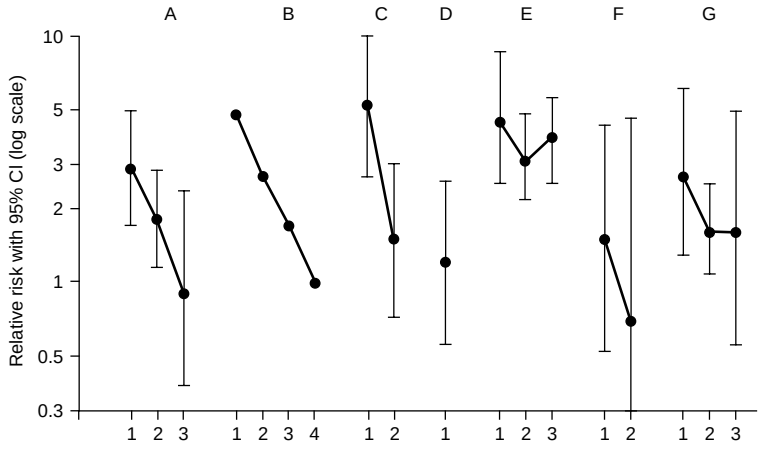

Fig. 10. Cerebral thromboembolism among users and non-users of oral contraceptives (OC) in seven recent studies. From (period of recruitment covered is given in brackets):
A.[167] 1. ethinyestradiol 0.05mg combined OC
2. ethinylestradiol 0.03-0.04mg combined OC
3. progestogen-only OC
B.[178] 1. ethinylestradiol 0.05mg combined OC
2. ethinylestradiol 0.03-0.04mg combined OC
3. ethinylestradiol 0.02mg combined OC
4. progestogen-only OC
C.[177] 1. ethinylestradiol 0.05mg combined OC
2. ethinylestradiol <0.05mg combined OC
D.[179] 1. ethinylestradiol <0.05mg combined OC
E.[182] 1. ethinylestradiol ≥ 0.05mg combined OC
2. ethinylestradiol <0.05mg combined OC with levonorgestrel or norethisterone progestogen
3. ethinylestradiol <0.05mg combined OC with desogestrel or gestodene progestogen
F.[180] 1. ethinylestradiol 0.05mg combined OC
2. ethinylestradiol 0.03-0.04mg combined OC
G.[183] 1. ethinylestradiol ≥0.05mg combined OC
2. ethinylestradiol <0.05mg combined OC with levonorgestrel or norethisterone progestogen
3. ethinylestradiol <0.05mg combined OC with desogestrel or gestodene progestogen.
CI = confidence level.

relative risk of subarachnoid haemorrhages ranged between 0.9 and 2.0 in 7 of the 8 studies, but did not achieve statistical significance in any study. One study included only 4 cases of current use and found a relative risk of 6.5 (1.9 to 22.6).[186] The only study specifically analysing intracerebral haemorrhages found no increased risk.[143]

Cohort Studies

Results from cohort studies of stroke in oral contraceptive users are shown in table IX. Where the data have been analysed separately, findings for thrombotic and haemorrhagic stroke are given. Numbers of cases in prospective studies of stroke in oral contraceptive users have been relatively small. The RCGP study found a risk of cerebral embolism in current oral contraceptive users that was consistent with the case-control studies performed during this period.[124] This study also found the risk of subarachnoid haemorrhage in current users to be somewhat increased but not significantly so. Vessey et al.[60] noted in the 1981 report on mortality in the Oxford/FPA Cohort that the RCGP data would have predicted 6.7 deaths from subarachnoid haemorrhage during the 142 210

Table VIII. Case-control studies of haemorrhagic stroke in oral contraceptive (OC) users

Date and source of cases	No. of cases (OC users)	Odds ratio (95% CI)	Reference
1968-90 UK population sample	95 (14)	1.5 (0.6-3.7)	Hannaford et al 1994[143]
1969-71 US hospital records; nonfatal	195 (44)	2.0 (1.0-3.8)	Collaborative Group 1973, 1975[170, 171]
1969-76 US population sample; fatal and non fatal	11 (4)	6.5 (1.9-22.6)	Petitti et al 1978[186]
1976 England and Wales; death certificates	109 (32)	1.4 (0.6-2.9)	Inman 1979[187]
1978 England and Wales; death certificates	168 (27)	0.9 (0.5-1.6)	Thorogood et al 1981[188]
1986-88 England and Wales; death certificates	296 (70)	1.1 (0.6-1.9)	Thorogood et al 1992[176]
1987-89 US population sample	103 (5)	0.9 (0.2-3.6)	Longstreth et al 1994[189]
1989-93 European hospital records	247 (41)	1.4 (0.8-2.3)	WHO Collaborative Study 1996[190]
1989-93 Developing country hospital records	821 (112)	1.8 (1.3-2.3)	WHO Collaborative Study 1996[190]
1991-94 US population sample; fatal and nonfatal	151 (21)	1.2 (0.5-2.6)	Petitti et al 1996[179]
1991-95 US population sample; nonfatal	100 (14)	1.4 (0.7-3.0)	Schwartz et al 1997[180]
1991-95 US population sample; nonfatal	173 (23)	1.1 (0.6-2.0)	Schwartz et al 1998[181]

CI = confidence interval.

women-years of follow-up, whereas only one death was seen, suggesting that the Oxford/FPA cohort was unusually healthy. It is also noteworthy that ischaemic stroke is rarely fatal in young women, so studies examining mortality from ischaemic stroke have tended to show less effect of oral contraceptives than those examining morbidity. This is exemplified by the relative risk of 1.0 for fatal cerebral thromboembolism[58] compared with 6.0 for all cerebral embolism[124] seen in the RCGP study. In a 25 year follow-up analysis of mortality in the RCGP cohort, the relative risk of death from any cerebrovascular disease among women who were currently using oral contraceptives or who had used them in the preceding 10 years was 1.9 (95% CI 1.2 to 3.1).[191]

Joint Effects with Other Risk Factors

In the report from the Collaborative Group for the Study of Stroke in Young Women that related to oral contraceptive use in the early 1970s, risks associated with both oral contraceptive use and hypertension were explored.[170] Compared with normotensive women not taking oral contraceptives, the odds ratio for thrombotic stroke in normotensive women taking oral contraceptives was 3.1 (95% CI 1.5 to 7.2), for women with severe hypertension not taking oral contraceptives 6.9 (3.3 to 14.5) and for women with severe hypertension taking oral contraceptives 13.6 (4.8 to 38.6). The equivalent figures for haemorrhagic stroke were 1.8 (0.8 to 4.4), 21.6 (11.1 to 42.3) and 25.7 (9.4 to 70.7). According to the WHO Collaborative Study, the odds ratio for ischaemic stroke in European normotensive women taking oral contraceptives was 2.7 (1.5 to 5.0). For women with a history of hypertension not taking oral contraceptives the odds ratio was 4.6 (2.4 to 8.8), and for women with a history of hypertension taking oral contraceptives 10.7 (2.0 to 56.6).[177] Very similar differentials were seen among women in developing countries. The equivalent figures for haemorrhagic stroke were 1.1 (0.6 to 1.8), 4.9 (3.0 to 8.2) and 10.3 (3.3 to 32.3), and again, although with a somewhat greater effect of history of hypertension alone, similar differentials were seen in the analysis for developing countries.[190] An approach to the possibility of undetected hypertension in the groups studied was the stratification according to whether or not BP had been checked that was adopted in the WHO Collaborative Study.[177,190] Odds ratios for ischaemic stroke and oral contraceptive use among those who had not had a BP check were 3.9 (Europe) and 3.8 (developing countries). For those had received a BP check, the equivalent figures were 3.8 and 1.9. For haemorrhagic stroke it was stated that BP checking made no difference to the odds ratios associated with oral contraceptive use.

Table IX. Prospective studies of stroke in oral contraceptive (OC) users

Date	OC status	Women-year follow-up (total)	No. of cases	Relative risk (95% CI)	Reference
Maternal Health Project, Puerto Rico					
1961-69	Never users	17 353	1 fatal stroke		
	Current users	12 155	2	0.7	17
UK Royal College of General Practitioners Study					
1968-72	Never users	42 306	0 CT		20
	Current users	34 875	4	-	
	Never users		1 SAH		
	Current users		2	3.8	
1968-76	Never users	91 521	3 fatal CTE		57
	Ever users	91 880	1	-	
	Never users		0 fatal SAH		
	Ever users		9	-	
1968-79	Never users	138 630	4 fatal CTE		58
	Current users	98 997	2	1.0 (0.8-1.2)	
	Ex-users	84 811	8	3.0 (0.7-12.0)	
	Never users		3 fatal SAH		
	Current users		6	3.2 (0.6-16.3)	
	Ex-users		11	4.5 (1.2-16.5)	
1968-79	Never users	129 593	0 CT		124
	Current users	98 551	10		
	Ex-users	78 142	4	-	
	Never users		1 CE		
	Current users		8	6.0 (1.4-25.6)	
	Ex-users		6	0.9 (0.1-11.1)	
	Never users		11 SAH		
	Current users		11	1.7 (0.8-3.8)	
	Ex-users		16	2.1 (0.9-4.6)	
Oxford/Family Planning Association Study					
1968-74	Never users	34 035	1 CTE		22
	Current users	21 794	3	-	
	Never users		2 SAH		
	Current users		2	-	
1968-80	Never users	62 532	1 fatal stroke		60, 61
	Ever users	79 678	2	1.3	
1968-87	Never users	118 671	3 fatal stroke		62
	Ever users	152 597	7	1.4 (0.3-8.5)	
Walnut Creek Contraceptive Drug Study					
1968-77	Never users	37 913	15 CTE		24
	Current users	20 134	3	2.2 (0.4-10.3)	
	Ex-users	19 118	11	1.2 (0.5-2.8)	
	Never users		2 SAH		
	Current users		5	10.1	
	Ex-users		1	2.3	
Puget Sound Health Cooperative Study					
1977-82	Non-users	167 901	7 stroke		27, 63
	Current users	37 807	1	0.9 (0.1-6.4)	

Table IX. Contd

Date	OC status	Women-year follow-up (total)	No. of cases	Relative risk (95% CI)	Reference
Nurses Health Study					
1976-84	Never users	484 096	185 stroke		23
	Current users	22 376	4	-	
	Ex-users	415 488	93	1.0 (0.8-1.3)	

CE = cerebral embolism; **CI** = confidence interval; **CT** = cerebral thrombosis; **CTE** = cerebral thromboembolism; **SAH** = subarachnoid haemorrhage.

Joint effects of oral contraceptive use and hypertension on risks of stroke may have diminished in importance as oral contraceptives ceased to be prescribed to women with elevated BP. However, effects of oral contraceptive use and cigarette smoking continues to be an area of concern. Again, in the report from the Collaborative Group for the Study of Stroke in Young Women, compared with nonsmoking women not taking oral contraceptives, the odds ratio for thrombotic stroke in nonsmoking women taking oral contraceptives was 3.2 (95% CI 1.6 to 6.4), for heavy smokers not taking oral contraceptives 1.5 (0.8 to 2.6) and for heavy smokers taking oral contraceptives 6.1 (2.9 to 13.1).[170] The equivalent figures for haemorrhagic stroke were 1.2 (0.5 to 2.9), 2.6 (1.5 to 4.5) and 7.6 (3.5 to 16.7). These findings suggested that the joint effects of oral contraceptive use and heavy cigarette smoking might be somewhat greater than the sum of the independent effects, particularly with regard to haemorrhagic stroke. In a study restricted to cases of subarachnoid haemorrhage, Petitti and Wingard[186] found that the relative risk for subarachnoid haemorrhage for women who used oral contraceptives and smoked was 21.9 (95% CI 8.5 to 56.2), the independent risks being 6.5 and 5.7, respectively, again suggesting that the joint effects of oral contraceptive use and heavy cigarette smoking might be greater than the sum of the independent effects. However, as described above, the relative risk of 6.5 for oral contraceptive use and subarachnoid haemorrhage was unusually high in this study. With regard to the paired population-based studies undertaken in the early 1990s in California and Washington State, US, in the California Kaiser Permanent Medical Care Program Study, adjusted analyses stratified by cigarette smoking returned odds ratio of ischaemic stroke for oral contraceptive use compared with non-use among nonsmokers of 1.3 (95% CI 0.6 to 3.1) and among smokers 0.7 (0.27-3.3).[179] The equivalent figures for haemorrhagic stroke were 0.8 (0.4 to 1.7) and 3.6 (0.95 to 13.9), so again there was the suggestion of a joint effect of oral contraceptive use and cigarette smoking with regard to haemorrhagic stroke. In the analysis of the Washington State study,[180] type of stroke was not distinguished, but the odds ratio for total stroke and oral contraceptive use among nonsmokers was 1.7 and among smokers 0.5. In the joint analysis of these two studies, there was no evidence of modification of risk among oral contraceptive users by cigarette smoking,[181] neither was there any modification according to age or BMI. According to the WHO Collaborative Study, among European women and compared with nonsmokers not using oral contraceptives, odds ratios for ischaemic stroke in smokers not using oral contraceptives were 1.2 (95% CI 0.7 to 2.1); in nonsmokers using oral contraceptives 2.1 (1.03 to 4.5); and in smokers using oral contraceptives 7.2 (3.2 to 16.1).[177] Similar differentials were seen between women in developing countries. The equivalent figures for haemorrhagic stroke were 1.2 (0.6 to 2.4), 2.10 (1.5 to 3.0) and 3.1 (1.7 to 5.8)[190] and, again, similar differentials were seen between women in developing countries. Therefore, in the WHO Collaborative Study, there was less of a suggestion that risks of haemorrhagic stroke might be unduly augmented by the joint effects of oral contraceptive use and cigarette smoking.

The possibility has also been raised that there may be joint effects between oral contraceptive use and a history of migraine. In the report from the Collaborative Group for the Study of Stroke in Young Women, compared with women without a history of migraine not taking oral contraceptives, the odds ratio for thrombotic stroke in women without such a history taking oral contraceptives was 4.9 (95% CI 2.9 to 8.3), for women with such a history not taking oral contraceptives 2.0 (1.2 to 3.3) and for those with a history taking oral contraceptives 5.9 (2.9 to 12.2). The equivalent figures for haemorrhagic stroke were 2.2 (1.3 to 3.6), 1.8 (1.2 to 2.7) and 2.6 (1.2 to 5.5).[170] A more marked differential was seen with regard to ischaemic stroke in the Paris hospital-based study of Tzourio et al.[178] Compared with women without migraine not using oral contraceptives, women without migraine using oral contraceptives had an odds ratio for ischaemic stroke of 3.5 (1.5 to 8.3), women with migraine not using oral contraceptives 3.7 (1.5 to 9.1) and women with migraine using oral contraceptives 13.9 (5.5 to 35.1). In the recent joint case-control analysis of population-based studies in the US the only suggestion of joint effect between oral contraceptive use and the presence of any risk factor was with a history of migraine. Odds ratios for oral contraceptive use and ischaemic stroke without and with such a history were 0.88 (0.44 to 1.76) and 2.08 (1.19 to 3.65) and for oral contraceptive use and haemorrhagic stroke 1.00 (0.46 to 2.19) and 2.15 (0.85 to 5.45).[181]

Effects of Duration of Current Oral Contraceptive Use

There are no reports of increased risk of stroke with increasing duration of current use of oral contraceptives, but neither does there appear to have been any reliable analyses of this issue. By analogy with MI, it seems unlikely that duration of current use has any major effect.

Effects of Past Use of Oral Contraceptives

Epidemiological studies do not suggest any increased risk of thrombotic stroke in women who have stopped using oral contraceptives, so any risk in current users probably disappears on cessation of therapy. This is illustrated by findings from the RCGP study in which the relative risk of cerebral embolism was 6.0 (95% CI 1.2 to 16.5) in current users and 0.9 (0.1 to 11.1) in ex-users.[124] There may even be a reduced risk in ex-users, as reported by one of us (Lidegaard[167]), who found a significantly decreased risk of thrombotic stroke of 0.5 (0.4 to 0.7). However, there has been some suggestion of an increased risk of subarachnoid haemorrhage in ex-users of oral contraceptives, again apparent in the RCGP study,[58,143] but not in the major case-control studies.[171,187] It is noteworthy, however, that the increased risk of death from cardiovascular disease detected by the Nurses' Health Study in ex-users who had previously used oral contraceptives for 10 years or more was associated with a relatively high proportion of deaths from stroke (9 out of 19 women), although the type of stroke was not distinguished.[133]

Variation in Risk with Oral Contraceptive Formulation

The possibility that the risk of thrombotic stroke varied with oral contraceptive estrogen dose was first raised in the analysis of adverse drug reaction reports of Inman et al.[76] No such association was found, however, in the report from the Collaborative Group for the Study of Stroke in Young Women,[171] but in the Oxford/FPA Study[192] there were no strokes during 9100 women-years of observation, relating to oral contraceptives containing estrogen 0.03mg or less, compared with 13 strokes during 39 400 women-years relating to higher dose formulations. Strong support for a link between estrogen dose and risk of thrombotic stroke was provided by a study in women in Denmark in which it was found that progestogen-only pills did not confer any increased risk, 0.03 to 0.04mg estrogen pills conferred a relative risk of 1.8 whereas oral contraceptives with estrogen 0.05mg increased the risk 2.9 times (fig. 10).[167] These trends have been confirmed in 3 other more recent studies (fig. 10).[177,178,183] Further support for an effect of oral contraceptive estrogen dose comes from the national incidence study by one of us (Lidegaard[138]), who analysed discharge diagnoses from

Danish hospitals between 1980 to 1993. Between 1980 and 1986 women in the age range 20 to 35 years had more attacks than men in the same age range, and after 35 years of age, fewer attacks. After 1987 the sex difference in the younger age group ceased to be significant. There was a significant fall in the incidence rate of cerebral thromboembolic attack throughout the duration of the study in 20 to 35 year old women but not men. Overall, available data suggests that oral contraceptives containing more than 0.05, 0.05 and 0.03 to 0.04mg estrogen are associated with odds ratios of 8 to 10, 2 to 4 and 1.5 to 2.5, respectively.[167]

No evidence for a link between estrogen dose and haemorrhagic stroke was found either in the Collaborative Group Study[171] or in the mortality study of Inman.[187]

The influence of the progestogen dose has been less investigated than has the impact of the estrogen dose. Rates of cerebrovascular disease in the RCGP study were 0.4, 0.7, 1.3 per 1000 women-years in users of oral contraceptives containing norethisterone acetate 1, 3 and 4mg in combination with ethinylestradiol 0.05mg.[135] The associated rates of hypertension were 8.2, 12.3 and 13.9 per 1000 women-years, respectively.[193] This differential in risk of stroke was also found in an analysis of adverse drug reaction reports by Meade et al,[77] and the dose relationship also appeared to apply to the progestogen, levonorgestrel. The effect of norethisterone acetate dose was still apparent in the analysis of RCGP study data by Hannaford et al.,[143] who found an increasing relative risk of stroke (thrombotic and haemorrhagic combined) with increasing doses of norethisterone acetate: 2.6, 3.6 and 6.7 for pills with norethisterone acetate 1, 2 and 4mg, respectively. Possible effects of progestogen type on stroke risk will be considered in section 5.

Summary

There is strong evidence for an increase in risk of thrombotic stroke in women taking older oral contraceptive formulations. This risk appears to have diminished with the introduction of formulations containing lower estrogen doses: the relative risk now appears to be below 2.0. In accord with this, a recent meta-analysis of 16 studies of ischaemic stroke in oral contraceptive users found an odds ratio of 1.93 (1.35-2.74) for low estrogen dose formulations in population-based studies controlling for cigarette smoking and hypertension.[194]

The risk of intracerebral haemorrhage is not increased by oral contraceptives. The relative risk of subarachnoidal bleeds is between 1.5 and 2 for high estrogen-dose oral contraceptives, but this increase is not statistically significant. The risk of subarachnoidal bleeds with low-dose pills may be less than 1.5.

The incidence rates of the different types of stroke increases nearly exponentially with age. Among young women, the absolute risk of thrombotic strokes is 1 to 2 per 100 000 per year. Although the relative risk (odds ratio) is unaffected by age, the absolute increase in risk of cerebral thrombosis by use of oral contraceptives is about 10-times higher for a women at 40 years than for women at 20 years. The absolute risk attributable to oral contraceptive use is 2 to 4 per 100 000 per year at 20 years, and about 10 times as much among women 20 years older. This implies that even though the risk of cerebral thrombosis may be increased significantly by use of oral contraceptives, the absolute risk for a young healthy user of oral contraceptive use remains very low.

2.4 Relative Impact of VTE, MI and Stroke

Although VTE is by far the most common of the occlusive vascular diseases in young women, the low case fatality rates make it the least serious. Case fatality rates have been estimated as 1 to 2% for VTE, 5 to 30% for stroke and 25-50% for acute MI.[7,29,195] Thus, in terms of numbers of deaths, a 0.5-fold increase in MI risk is at least as important as a two-fold increase in risk of death from VTE. This relatively low mortality from VTE was apparent in the RCGP study.[58] Group-specific excess mortality was 9.7 per 100 000 women-years for cerebrovascular disease, 9.7 per 100 000 women-years in the category nonrheumatic heart disease and hypertension (mainly MI), but only 2.5 per 100 000

women-years for VTE. Equally, over the 20 years into the Oxford/FPA Study no women had died of venous thrombosis or embolism whereas 37 had died from other circulatory diseases.[62] The relative importance of arterial disease is also illustrated in the WHO figures for cardiovascular mortality among women in the reproductive age range presented by Farley et al.[195] At all ages death rates from stroke exceeded those from VTE, as did those from MI in older age groups. Among smokers, death from MI became more important than VTE in younger women (≥25 years) as well.

In this respect, consideration of current population-based mortality and morbidity figures for occlusive vascular disease is of importance. In non-pregnant women below 30 years of age in Denmark incidence rates of VTE, MI and stroke are 108, 6 and 46 per million, respectively, whereas the equivalent figures for women aged 30 to 44 years are 231, 114 and 222 per million.[29] The case fatality rate among women with VTE is estimated at between 0.6 to 1.7% whereas for MI it is between 18 to 25%. It may be estimated that the overall mortality rate from MI in non-pregnant women aged less than 30 is 83% higher than it is for VTE, and the equivalent figure for women aged 30 to 44 is 700% higher. Moreover, 33% of women surviving a MI have significant disability, compared with 5% of women who have survived a VTE. Clearly, in younger women, any impact of oral contraceptive use on risk of MI is likely to be as important, with regard to morbidity and mortality, as impact on VTE. In older women, impact on MI far outweighs that on VTE. Incidence and mortality from occlusive vascular disease in women in Denmark in the age ranges 15 to 29 and 30 to 44 years are shown in figure 11.

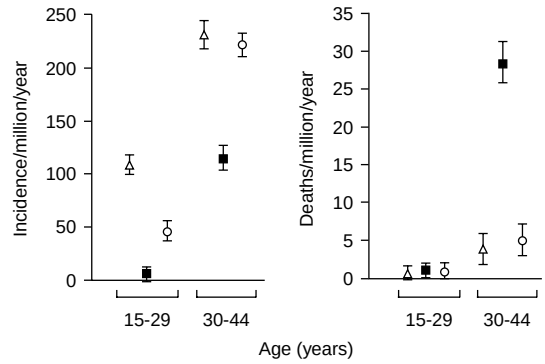

Fig. 11. Morbidity and mortality for venous thromboembolism (open triangles), myocardial infarction (closed squares) and cerebral thromboembolic attack (open circles) in young women in Denmark 1980 to 1993 in the age ranges 15 to 29 and 30 to 44 years (rate point estimates and 95% confidence intervals are shown).[29]

3. Vascular Pathology Associated with Oral Contraceptive Use

In a letter to the British Medical Journal in 1965, describing atypical coronary occlusion in an oral contraceptive user, GR Osborn wrote, '[t]he possible danger of the pill to coronary arteries could soon be established by adequate microscopic examination of the hearts of women of childbearing age who die from coronary disease'.[106] No such programme was ever undertaken. As Basdevant and colleagues[196] commented in 1980, '[t]his can be explained by the obvious technical problems and by the fact that incidents of mortality permitting anatomical and pathological verification are comparatively rare' [translated]. Nevertheless, such problems could have been overcome. The lack of a formal programme of investigation into the structural changes accompanying occlusive vascular disease in oral contraceptive users, or indeed into any direct vascular effects of contraceptive steroids, remains a major shortcoming in research into oral contraceptive safety. Instead, a proliferation of studies – frequently underpowered – of oral contraceptive effects on humoral factors has taken the place of intensive investigation into vascular pathology in oral contraceptive users. The sporadic histopathological and cardiovascular studies in the literature are, however, surprisingly consistent and suggest distinct pathogenetic mechanisms, specific to oral contraceptive steroids.

3.1 General Pathology of Occlusive Vascular Diseases

Vascular disease in oral contraceptive users has usually been interpreted in relation to the general

features of the most commonly encountered occlusive disorders: thrombosis and atherosclerosis. However, these are end-stage conditions that appear to result, in some cases after a protracted period, from dysfunctional interactions between the vascular endothelium and the haemostatic system.[197-199] In attempting to elucidate the pathology and aetiology of vascular disease in oral contraceptive users, it may therefore be more useful to consider the evidence in this wider context rather than to attempt to limit discussion to more conventional reference points. This section, nevertheless, begins with overviews of aspects of the general pathology of vascular occlusion, but with an emphasis on the role of endothelial dysfunction in these conditions, and on those aspects that have been of particular relevance to vascular disease in oral contraceptive users.

3.1.1 Endothelial Function and Dysfunction

The endothelium mediates the balance between patency and occlusion of the vascular lumen, the former necessary for normal blood flow and the latter for prevention of blood loss. The specific roles of the normal endothelium are listed in table X.[197,198,200]

Normal endothelium combines anticoagulatory, fibrinolytic and antiplatelet aggregating activities. Endothelial involvement in normal haemostatic function will be considered in detail in section 4.8. Importantly, the endothelium acts as a barrier between circulating platelets and the underlying material of the elastic lamina which, when exposed by endothelial disruption, becomes a potent stimulus to platelet aggregation. The endothelium also controls inflammatory reactions at the vessel wall through inhibition of leucocyte adhesion to the endothelium by nitric oxide (NO) and prostacyclin.

Table X. Functions of the vascular endothelium

Maintenance of blood fluidity
Inhibition of platelet adhesion
Protection against thrombosis
Control of vasodilation and constriction
Control of vascular smooth muscle cell proliferation
Uptake of macromolecules
Lipoprotein metabolism

Endothelial synthesis of NO from L-arginine has a major role in control of vascular tone,[201] counterbalancing the constrictor tone imposed by the sympathetic nervous system.[202] Inhibition of NO production then leads to vasoconstriction and hypertension. Control of vascular tone by endothelially-derived NO appears to be of primary importance in arteries and arterioles. In contrast to the arterial endothelium, venous endothelium, although capable of releasing NO, does not do so under basal conditions. Moreover, veins show little NO release in response to platelet-derived mediators, venous constriction being the dominant effect associated with aggregating platelets. These differences in NO release between arteries and veins could contribute to the greater tendency for veins to occlude.[198]

The endothelium is closely involved in the maintenance of vascular structural integrity, being responsible for the synthesis of components of the underlying elastic lamina, which itself modulates the growth, proliferation and function of the endothelium.[203] A further structural role involves endothelium-dependent inhibition of the proliferation of underlying smooth muscle cells.[204] This may be mediated by the various vasodilator, antiplatelet and antithrombotic agents elaborated by the endothelium, whose effects predominate in healthy basal endothelial function. However, where the basal endothelial state has been disrupted and there is endothelial proliferation, the inhibitory effect of the endothelium on smooth muscle cell proliferation can change to one of stimulation.[204] The endothelium also controls access to the subendothelial space by macromolecules. Rapid, unidirectional transport of such molecules in vesicles across the endothelium is effected by transcytosis.[205,206] Nonselective permeability depends on movement of molecules through the cellular interstices. This will, in turn, depend on the physical characteristics of the endothelial cells which may be appreciably modified in inflammatory states, leading to greater nonselective permeability.[207] Of importance with regard to the selective permeability of the endothelium is the observation that receptor-mediated up-

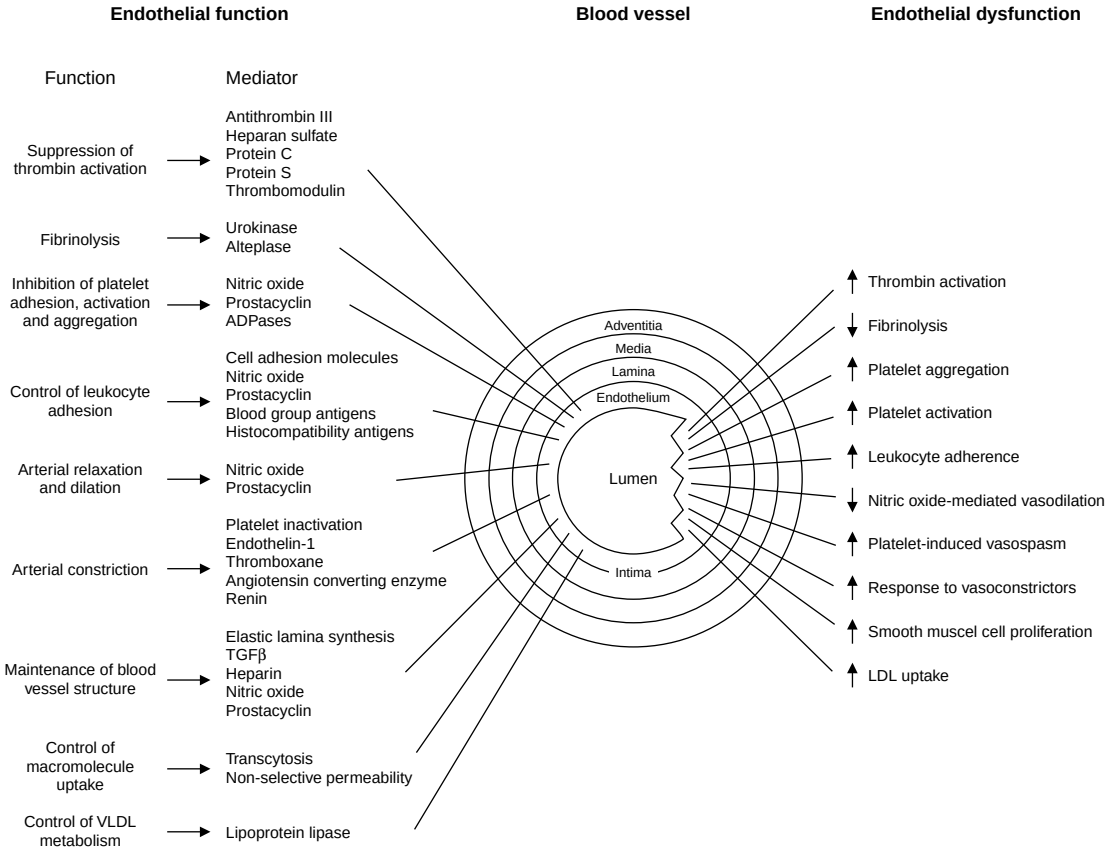

Fig. 12. Functions of the vascular endothelium and their principal mediators (left-hand register) and characteristics of endothelial dysfunction (right-hand register). **LDL** = low density lipoprotein; **TGF** = transforming growth factor; **VLDL** = very low density lipoprotein.

take of LDL cholesterol is down-regulated in endothelial cells, indicating that the normally functioning endothelium acts as a metabolic as well as a physical barrier to uptake of LDL cholesterol by the vessel wall.[208]

Between the two extremes of normal endothelial function with maintenance of a patent vascular lumen, and the effects of endothelial damage with promotion of vascular occlusion, lie a range of dysfunctional states which may, in the absence of life-threatening mechanical damage, bias endothelial activity in favour of venous occlusion. These more subtle states of dysfunction may ultimately be expressed in clinically manifest occlusive vascular disease. The effects of endothelial dysfunction may be inferred from the actions of a normally functioning endothelium described above. The principal change that has been characterised is attenuation of NO-mediated vasodilatory response. Deterioration in endothelial function appears to be related to the presence of risk factors for arterial disease.[209] The current list of risk factors for which there is evidence for such endothelial dysfunction includes elevated LDL cholesterol levels, hypertension, aging, cigarette smoking, menopause, diabetes mellitus and lack of exercise.[210] Processes contributing to normal endothelial function and the ways in which dysfunction in any of these processes can predispose towards vascular occlusion are illustrated in figure 12.

3.1.2 Venous Thrombosis and Thromboembolism

Venous thrombosis and embolisation of venous thrombi, though rare, are the most commonly reported major adverse effects of oral contraceptive use. 'Virchow's Triad' of disturbances in haemostatic factors in the blood, the vessel wall and blood flow still provides the core explanation for intralumenal thrombus formation, but the relative importance of these disturbances, their exact nature, and the minimum disturbance necessary for thrombus formation remains controversial. The rare occurrence of venous thrombosis, even in those with predisposing conditions, suggests that genetically-determined susceptibility is of particular importance.

General Features

Conditions associated with venous thrombosis include surgery and postsurgical recovery, pregnancy and the puerperium, cardiac disease, malignancy and immobilisation.[211] Increased activation of the coagulation system, damage to the vessel wall, or blood stasis feature to some degree in each of these conditions. More than 90% of venous thrombi originate in the deep veins of the calf.[212] If untreated, 15 to 25% of these progress to the proximal veins of the lower limb, and this carries about a 5% risk of pulmonary embolism, the most frequent serious consequence of venous thrombosis.[213-215]

Humoral Factors

Evidence favouring a primary role for disturbances in haemostatic factors in the blood has been explored in detail by Thomas[216] and the main points of his argument are summarised here. Although low rates of blood flow characterise areas in which venous thrombi tend to form,[217] stasis alone cannot initiate thrombus formation, as evidenced by the observation made by Hewson over 200 years ago[218] that blood in isolated veins will not clot for many hours. Nevertheless, stasis combined with hypercoagulability is sufficient for thrombus formation, and this appears to be independent of damage to the vessel wall.[219,220] The permissive effect of stasis may relate to the rapid exhaustion of local endothelial anticoagulatory mechanisms. A further contributing factor appears to be the decreased fibrinolytic factor levels and increased antifibrinolytic factor levels seen in states of venous stasis in patients with thrombosis.[221,222]

Damage to the Vessel Wall

The role of damage to the vessel wall in the pathogenesis of venous thrombosis is less certain. Experimental studies indicate that crush injury to veins, though accompanied by platelet adhesion and white cell infiltration at the damaged area, does not result in fibrin deposition.[223] Moreover, in one study of postmortem material, no evidence of prior intimal damage to the vessel wall was found in association with valve pocket thrombi.[224] The authors considered that endothelial damage was an appropriate model for arterial thrombosis with its high platelet content, but that stasis was the principal factor with regard to venous thrombus, with its fibrin-enmeshed red and white cells and low platelet content.

Endothelial Dysfunction

There remains the possibility that more subtle changes in endothelial function, might contribute to intra-lumenal thrombus formation.[225] Leucocytes adhere to the endothelium in areas of blood stasis and migrate into the subendothelial space. In so doing they disrupt endothelial continuity and initiate a reaction comparable to that seen in inflammation. Thus, invasion of white cells during stasis could lead to vessel wall damage sufficient to promote thrombosis.[226-228] Leukocyte adhesion could also contribute to a hypercoagulable state, by promoting platelet accumulation and activation, and by releasing procoagulant material. Further evidence for involvement of endothelial dysfunction in thrombus formation comes from the observations that over 50% of patients with idiopathic DVT have hypercholesterolaemia.[229] It is now well-established that elevated plasma LDL cholesterol levels can promote endothelial dysfunction.[230,231]

Summary

The current weight of evidence supports a general explanation for venous thrombus formation that involves activation of the coagulation system

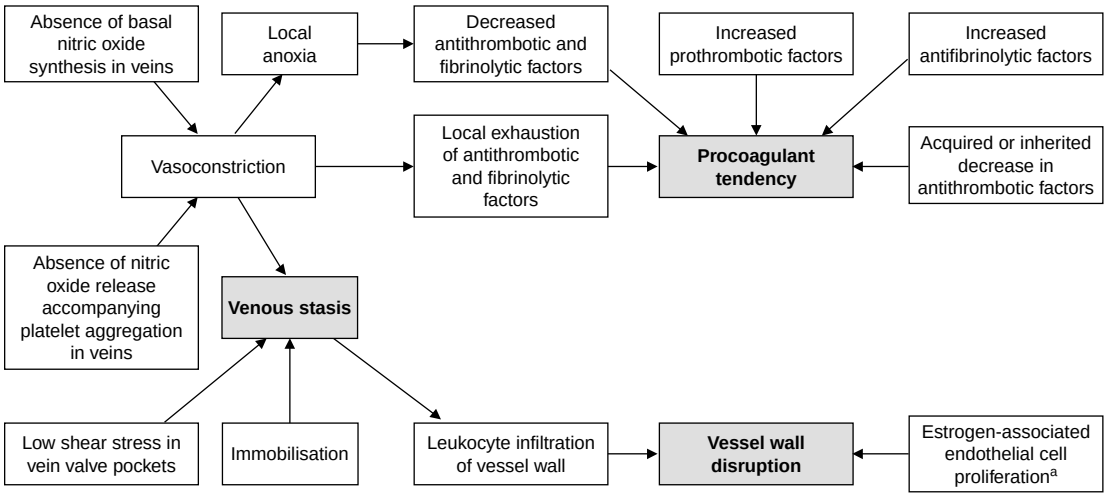

Fig. 13. Interrelationships between factors involved in the aetiology of venous thromboembolism.
a See section 3.2.1.

in an area of venous stasis. The contribution of endothelial damage or dysfunction remains uncertain. The haemodynamic, anticoagulatory and fibrinolytic characteristics of areas of blood stasis favour thrombus stabilisation and propagation. It is noteworthy that several properties of normal veins contribute to a predisposition towards formation of occlusive thrombus. Valve pockets provide an area of relative stasis where local exhaustion of anticoagulant and fibrinolytic factors could take place. Moreover, the absence of basal NO release or of release of NO in association with platelet aggregation in veins are likely to favour a constrictor tendency. Factors contributing to the pathogenesis of VTE and their interrelationships are summarised in figure 13.

3.1.3 Acute MI

The two principal sources of clinical information regarding the aetiology of acute MI are coronary angiography in those who survive an acute MI, and autopsy (including angiographic examination of the coronary arteries) in those who do not. In the majority of cases, angiography reveals irregular stenoses and severe narrowing of one or more major coronary arteries.[232,233] On necropsy, this occlusion is generally found to be caused by thrombus, formed in association with an atheromatous plaque.[234,235] A significant minority of patients who have survived a MI have normal coronary angiograms, or atypical angiograms showing only moderate, regular lumenal narrowing, in contrast to irregular and severe narrowing. Such cases may be important with regard to the aetiology of MI in oral contraceptive users and are reviewed in detail in this section.

Coronary Artery Thrombosis and Atherosclerosis

Whatever its precipitating factor or subsequent development, coronary artery occlusion is almost invariably associated with thrombus formation. The aetiology of arterial thrombus differs from that of venous thrombus in that stasis is of relatively minor importance, whereas endothelial damage or dysfunction is critical. Arterial thrombus is characterised by a relatively platelet-rich fibrin matrix with inclusion of white but not red blood cells. This relates to the relatively high flow and shear rates in the artery, particularly in areas where there is lumenal narrowing by atherosclerotic plaque. High flow rates ensure a plentiful supply of platelets and the shear stress may promote platelet aggregation.[236] High flow rates diminish incorporation of red cells into the thrombus but the shear stress may

damage red cells, resulting in release of adenosine diphosphate (ADP), which then acts as a vasoconstrictor in areas of endothelial damage. Platelet accumulation in areas of endothelial damage or dysfunction will not be the only factor in thrombus formation and progression. Disturbances in the anticoagulatory and fibrinolytic properties of the endothelium will contribute. Moreover, the balance between vasoconstriction and vasodilation will be disrupted in favour of the former and vasospasm will favour local accumulation of thrombus on any area of damaged endothelium.

MI is most commonly caused by an occlusive thrombus formed at the site of an atherosclerotic lesion.[237,238] This has led to considerable, and sometimes exclusive emphasis on the development of the atherosclerotic plaque in explanations for the aetiology of MI. However, it must be kept in mind that atherosclerosis is merely one particularly dramatic and well characterised consequence of endothelial damage and dysfunction. The latter may themselves be sufficient for arterial thrombus to form or vasospasm to occur without the formation of typical atherosclerotic lesions.

The pathogenesis of atherosclerosis has been reviewed extensively elsewhere.[239-242] In brief, the most-widely held hypothesis for atherogenesis would have the lesion develop out of an inflammatory response to repeated endothelial injury. Atherosclerotic plaques tend to develop in the proximal regions of the coronary arteries, close to branch points, which are sites of turbulent blood flow and blood stasis. Progression of vascular damage in lesion-prone areas begins with a normal protective response involving expression by the dysfunctional endothelium of adhesion molecules, growth promoting substances and factors capable of inducing activation of the coagulation cascade, fibrin deposition, intimal smooth muscle cell proliferation and migration and matrix deposition. Pathology in the area of endothelial damage then develops in the face of chronic exposure to agents which caused or sustain this damage, such as toxins in tobacco smoke, the increased shear stress associated with hypertension, and increased LDL cholesterol levels and oxidatively damaged LDL. LDL, the principal cholesterol-carrying lipoprotein in humans, can enter the vessel wall directly through the damaged endothelium or by transcytosis. Reactive oxygen species, generated in the intima by leukocytes and endothelial and smooth muscle cells, modify LDL and this oxidised LDL can itself be toxic, inducing further endothelial damage. More importantly, LDL oxidation accompanies monocyte activation and differentiation, during which the monocyte is transformed into an active macrophage, which takes up oxidised LDL but does not down-regulate its receptor as cholesterol accumulates. Consequently, the macrophage develops into the lipid-laden 'foam' cell. Foam cell accumulation results in the characteristic fatty streak, one of the earliest identifiable lesions in atheroma. Further oxidative stress within the intima causes foam cell necrosis and the accumulation of extracellular lipid. The end-stage of atheromatous plaque progression involves rupture of a previously noncritical atherosclerotic stenosis (i.e. one occupying 20 to 70% of lumenal diameter). It is this plaque rupture that is likely to be of primary importance in the aetiology of MI, rather than occlusion by incursion of atherosclerotic plaque into the artery lumen.[243] Release of plaque material and sloughing off of the plaque endothelial cap following rupture results in activation of the coagulation cascade, loss of the antithrombotic influences of the endothelium and thrombus formation. Mural thrombi may become incorporated into the atheromatous lesion, further stimulating underlying smooth muscle cell proliferation and eventual replacement by collagenous matrix, or they may progress to become an occlusive thrombus.

MI with Angiographically Normal Coronary Arteries

Post-infarction angiography shows that some cases of MI do not involve the typical irregular coronary wall profile and severe stenosis that is taken to signify the presence of atherosclerosis. With regard to oral contraceptive users who have experienced a MI, such atypical angiography assumes particular importance. Cases of MI with an-

giographically normal coronary arteries are therefore considered in detail here, as are criteria for a negative coronary angiogram and the various interpretations that can be placed on such an angiogram.

In angiographic studies, atherosclerotic coronary disease is usually evident in an uneven coronary artery profile which extends beyond the site of a single lesion and involves more than 70% occlusion at one site or more.[244,245] The term 'angiographically normal' is used imprecisely, but generally refers to: (i) lumenal stenoses of less than 25%; (ii) somewhat larger stenoses which are isolated and show no accompanying vessel wall irregularities, or (iii) no detectable stenosis. It has at times been assumed that such normal angiography makes the presence of atherosclerosis unlikely.

However, as described above, coronary occlusion results not so much from the space occupied by raised plaque as by plaque fissure and thrombosis. Plaques that fissure need not occupy a significant proportion of the vascular lumen. In fact, it has been estimated that about half of all coronary events are associated with plaques that occupy less than 50% of the vascular lumen.[246] Thus, a negative angiogram can be associated with atherosclerotic lesions that are insufficiently raised for angiographic detection but which are nevertheless unstable.

Other factors obscuring the presence of atherosclerosis on angiography include the fact that atherosclerosis tends to be relatively diffuse so there may be appreciable vascular narrowing adjacent to an apparent focal lesion. Use of such an adjacent region as a reference in angiography then results in underestimation of lumenal narrowing. Another important factor determining a negative angiogram may be vascular remodelling, which can restore normal lumenal diameter in an area of atherosclerosis.[247] Areas of non-occlusive atheromatous disease that can act as a focus for thrombus formation may therefore be present, and dissolution of the occluding thrombus between infarction and angiography may then result in a normal angiographic profile.

Beyond questions over the ability of angiography to distinguish clinically important atherosclerotic lesions, it is well recognised that there may simply be misinterpretation of the angiogram, or failure of the angiogram to distinguish an occlusion that is, in fact, present.[248] It is also appreciated that the extent of coronary atherosclerosis can be substantially underestimated by coronary angiography. In one recent series of 884 patients with angina pectoris or MI only 6.8% of angiographically normal coronary artery segments were found to be free of atherosclerosis as assessed by intravascular ultrasound.[249]

Therefore, whereas a positive angiogram probably signifies the presence of atherosclerosis, a negative angiogram by no means excludes it. However, there are several considerations which make MI with normal angiography an issue of concern. False negative angiography cannot explain the occasional postmortem studies showing absence of atherosclerosis and, importantly, neither can it explain the distinctive risk factor profile of those who have experienced a MI but who have normal coronary angiograms.

Natural History

MI without demonstrable coronary artery disease is uncommon, and most publications deal with case reports[250-273] or case series.[232,248,274-277] Prevalence rates of less than 1% of all cases of MI have been reported,[232,275] although other studies have found between 3 and 12%.[274,276-279] Incidence appears to be inversely related to age;[248] in one study, 45% of patients were less than 35 years of age.[276] Angina pectoris prior to the event is rare, although some cases have been reported.[250,253,280] The typical patient described in the cardiological literature is a young male smoker[278,281] or a young woman who is pregnant or taking oral contraceptives.[269,272,273,282] The time elapsed between MI and angiography in studies of patients with myocardial infarct with angiographically normal coronary arteries is extremely variable, extending to almost 15 years in one study.[282] At least several weeks is normal, so it has been argued that the association between MI and angiographically normal coronary arteries is an unproven combination.[283] However, histological evidence for ab-

sence of atherosclerosis has been obtained within two days of the infarction[265] and the shortest times reported between infarction and angiography are 3.7 to 12.5 hours.[271]

Patients who experience a MI but are subsequently found to have normal coronary angiography may have a better prognosis than those who have angiographic evidence of coronary obstruction.[273,276-278,280,282] This could simply reflect the fact that they have less severe atherosclerosis. Nevertheless, the distinctive risk factor profile apparent in such patients suggests a more basic difference. Glancy and colleagues[256] described the case of a woman with no history of angina pectoris, diabetes mellitus, hypertension, obesity, valvular heart disease, hypercholesterolaemia or a family history of premature coronary artery disease. Her only distinguishing features were a recent pregnancy and cigarette smoking. Of the 9 patients described by Khan and Haywood,[278] 8 were cigarette smokers but none had elevated serum cholesterol, triglycerides, glucose, uric acid or BP. In the case series and literature review of Rosenblatt and Selzer,[270] 15 of 25 patients with MI with angiographically normal coronary arteries had no known risk factors compared with none in a group of 16 such patients with abnormal angiograms. None of the former group had a history of angina pectoris whereas 10 of the latter group did. In the study of Raymond et al.,[282] patients with MI with a normal coronary angiogram had less family history of coronary artery disease, and less prevalent hyperlipidaemia, hypertension and glucose intolerance than comparable patients with abnormal coronary angiograms. The former group were significantly more frequently exposed to high levels of female sex hormones, either as a result of pregnancy or oral contraceptive use, and also showed a comparatively high prevalence of migraine. Cigarette smoking was more common in both groups than in the group with normal coronary angiography and no MI.

Mechanisms

Beyond the possibilities of false-negative angiography or angiographically undetectable atherosclerosis, a number of explanations have been put forward to account for MI in the absence of subsequent significant coronary occlusion on angiography or autopsy. These include thrombosis with subsequent dissolution of the thrombus,[284] coronary vasospasm,[285,286] embolisation with subsequent dissolution of the thrombus,[287] dissection or arteritis,[288] cardiotoxicity associated with cocaine abuse[289] or myocarditis.[290]

Impairment of the antithrombotic properties of the endothelium in a region of coronary endothelial dysfunction or damage could result in localised thrombus formation and thrombotic occlusion. Henderson and colleagues[291] described the case of an oral contraceptive user who had experienced a MI and who showed 50% obstruction of the proximal left anterior descending coronary artery on angiography 7 months after the event. The obstruction was smooth and fusiform, in contrast to the more typical rough, irregular appearance seen in cases of atherosclerosis. 38 months after the event there was no coronary occlusion apparent. Thrombus formation might also promote further changes predisposing to coronary occlusion by initiating fibroproliferative changes in the vessel wall as well as smooth muscle cell activation and migration.[292-294] Embolisation causing blockage of the coronary arteries can result from thrombus formation on damaged or infected cardiac valve or sinus tissue.[287] Such emboli are generally found in the distal regions of the epicardial coronary arteries, in contrast to the proximally-located thromboses associated with atherosclerotic plaque.[244] Emboli formed elsewhere in the circulation will be trapped in the pulmonary circulation before entering the coronary arteries.

In addition to providing a site for thrombus formation, the dysfunctional endothelium may also provide a focus for release of platelet-derived vasoconstrictors. An increased tendency to vasoconstriction at a site of endothelial dysfunction will also be apparent in an abnormal vasoconstrictor response to vasoactive agents, as has been demonstrated in coronary arteries that showed only minor lumenal irregularities on angiography.[295] Coronary artery spasm has been proposed as the cause

of acute MI in certain patients[248,284,296-302] and this possibility is strengthened by provocative testing with ergonovine.[282,297] Such testing has suggested a 30% prevalence of coronary artery spasm among patients with MI and normal coronary arteries.[277,282,284] Spasm alone might be sufficient to cause infarction or it might act as a focus for occlusive thrombus formation.[284,286,303] Nevertheless, the importance of spasm has been questioned, since patients with authenticated coronary spasm tend to have experienced prior angina pectoris, which is not generally a feature of MI with normal coronary arteries.[270,283]

Spasm or rapidly-dissolving platelet thrombus were proposed to account for rapid resolution of the presumed obstructions in the 3 events studied by Oliva and Breckenridge,[271] in which normal angiograms were found 3.7 to 12.5 hours after infarction. Platelet aggregates have been found in the coronary vasculature in cases of sudden death, although these were too small to cause infarction.[304] Moreover, platelet aggregates generally dissolve within 10 minutes,[305] and coronary artery occlusion for at least 25 minutes is necessary for infarction.[234] However the platelet disaggregation time in patients with MI could be prolonged.[306]

One explanation for the sustained coronary spasm necessary for infarction could be the development of a cycle of localised platelet aggregation, release of platelet vasoconstrictors, vasoconstriction and further platelet aggregation.[307] Endothelial damage would then be a critical factor since it would provide the site of platelet aggregation and increased platelet aggregability. Resolution of the event would then result in rapid platelet dissolution and angiographically normal coronary arteries. With regard to the findings of Oliva and Breckenridge,[271] it seemed unlikely that recanalisation or resolution of in situ thrombus would have occurred between infarction and angiography, since coronary emboli, occurring as a complication of cardiac catheterisation have been shown to take 5 to 8 weeks to resolve.[308]

Table XI. Characteristics associated with myocardial infarction with angiographically 'normal' coronary arteries

Stenosis, if detectable on angiography, insufficient to account for infarction damage
Prevalent in up to 12% of all survivors of myocardial infarction
Good prognosis compared with patients with abnormal angiography
Less frequently associated with previous angina pectoris, hypertension, hyperlipidaemia, diabetes mellitus or increasing age
More frequently associated with cigarette smoking or increased levels of female sex hormones
Aetiology most likely spasm or resolving thrombus associated with an area of endothelial damage or dysfunction

Summary

MI can occur in the absence of angiographic evidence for atherosclerotic disease. In these cases there may be no stenosis or atypical stenoses involving only limited narrowing of the coronary artery lumen or smooth isolated areas of narrowing. Although atherosclerosis is not precluded by these observations, cases tend to occur in younger individuals who smoke or who have been exposed to high levels of female sex steroids. Classic risk factors for atherosclerosis such as hypertension, hyperlipidaemia, diabetes mellitus or advanced age are less prevalent than expected and there is less previous angina pectoris reported. Typical features of MI in the absence of angiographic evidence for atherosclerosis are summarised in table XI.

3.1.4 Stroke

Ischaemic stroke, including lacunar stroke and TIA, involves occlusion of a cerebral artery, with consequent ischaemia or infarction of the tissues supplied.[309] Cerebral infarction is the commonest cause of stroke and atheromatous arterial disease with thromboembolism is the commonest cause of cerebral infarction. Atheroma is typically detected at branch points in the carotid and vertebral arterial supply to the brain, and thrombi formed on developed lesions in these areas may occlude the artery or embolise to distal sites. Atheromatous disease, once present, is likely to be widespread, regardless of whether its primary clinical manifestation is cerebral, cardiac or peripheral. TIA may be associated with either angiographically demonstrable

disease of the internal carotid artery or embolisation of thrombi formed in the aorta or the heart, possibly during atrial fibrillation.

The most common cause of primary intracranial haemorrhage is rupture of an aneurysm, which may be saccular, atherosclerotic or dissecting in origin. Bleeding into the brain is determined by the location of the lesion, and differential diagnosis between cerebral infarction and primary intracerebral haemorrhage generally requires computerised tomography or magnetic resonance imaging. Bleeding into the subarachnoid space usually results from rupture of an intracranial saccular aneurysm, which has developed on the medium-sized arteries at the base of the brain, probably as a result of atherosclerosis and hypertension.

Risk factors for cerebral infarction resemble those for MI, although hypertension assumes considerably more importance and dyslipidaemia less.[310] Risk of haemorrhagic stroke, including subarachnoid haemorrhage, is also highly dependent on BP and cigarette smoking. In contrast to ischaemic stroke, however, there appears to be a marked relationship with low serum cholesterol.[311] There is a continuous increase in risk with increasing BP in both sexes and at all ages. The risk of stroke doubles with every 7.5mm Hg rise in diastolic BP (DBP). In contrast to MI, there appears to be no gender difference in susceptibility to cerebral infarction.[310] Whether ischaemic stroke offers parallels to MI in the existence of a subgroup of cases in which there is no angiographically detectable stenosis of the carotid arteries does not appear to have been addressed. As described in section 3.2.1, when the pathophysiology of arterial occlusion in this subgroup of younger, generally cigarette smoking, oral contraceptive users is addressed, it is seen that similar lesions have been observed in the carotid and cerebral arteries to those found in other arterial systems. This suggests that there may indeed be a subgroup of cases of ischaemic stroke in which there is no apparent arterial stenosis on angiography.

3.1.5 Summary

Differing manifestations of occlusive vascular disease share certain features, in particular the possible contributions of endothelial dysfunction and activation of the haemostatic system. However, the differences that are seen are instructive. Venous thrombotic occlusion appears to have a requirement for inappropriate activation of the haemostatic system with venous stasis acting as a permissive factor. The role of endothelial damage or dysfunction is less well established. In contrast, endothelial damage appears to be the prerequisite for coronary artery occlusion. This is most dramatically seen in the most common cause of coronary occlusion, atherosclerosis. However, some cases of coronary occlusion may occur in the absence of the typical raised, lipid-rich lesions of atherosclerosis. In such cases endothelial dysfunction with thrombotic or vasospastic occlusion is the more likely cause. Thrombotic stroke may result from embolisation of thrombi formed in the heart or on atherosclerotic lesions in the arterial system. Haemorrhagic stroke is associated with breakdowns in vascular integrity, usually manifest in aneurysms in the cerebral vasculature. The importance of elevated BP in all forms of stroke suggests, however, that mechanical damage to the cerebral vasculature is of primary importance. The importance of damage to the endothelium in arterial as opposed to venous disease is also evidenced by the role of tobacco smoking, which is associated with release of vasotoxic agents into the pulmonary venous circulation. Smoking does not seem to contribute to venous thrombosis, although hypercholesterolaemia, with its attendant endothelial dysfunction may be a contributing factor. In contrast, disturbances in factors of the haemostatic system are of considerable importance in venous thrombosis and this is of particular interest with regard to the hereditary 'thrombophilias' which appreciably increase the risk of venous but not arterial disease, this despite the fact that thrombosis plays an important role in arterial occlusion.

In principal, any factor that might promote endothelial damage might be expected to promote vascular occlusion. Likewise, any factor that disturbs the haemostatic system in favour of an increased tendency to thrombosis (including endo-

thelial damage or dysfunction) might also be expected to promote vascular occlusion. There must then be a complex of relationships between the disturbances in endothelial function, haemostatic factors and the vascular and blood flow characteristics of the affected site which result in these distinctive pathologies of vascular occlusion.

3.2 Use of Contraceptive Steroids

3.2.1 Studies in Women

The few published reports concerning vascular pathology in oral contraceptive users may be divided into angiographic studies of coronary artery narrowing and autopsy studies, some of which have involved histological examination of the vasculature. The angiographic studies are described first since they concern only the coronary arteries and the atypical features that are revealed in oral contraceptive users may then be interpreted in terms of the distinctive vascular changes revealed by autopsy and histopathological studies.

Angiographic Studies

Vascular pathology of MI in oral contraceptive users has been studied largely by the indirect approach of coronary angiography. As described in section 3.1, there are a number of uncertainties inherent in interpretation of the angiographic profile and a negative angiogram need not indicate the absence of atherosclerosis. Such findings should be interpreted in relation to postmortem evidence, where available, and also to the risk factor profile of the individual concerned.

Early Case Reports and Case Series

In 1971, Dear and Jones[312] described two oral contraceptive users aged 27 and 37 years who survived an acute MI. Electrocardiographic changes typical of acute anterior infarction were seen, and a single occlusion of the anterior descending coronary artery was evident on angiography. The pattern of angiographic changes was not typical of that seen with generalised atherosclerosis. Such isolated segmental occlusions were also reported by Waxler and colleagues[313] in 3 oral contraceptive users who survived a MI. In one there was an isolated smooth segmental obstruction of the left circumflex coronary artery, whereas in the other two similar isolated smooth segmental obstructions were present in the anterior descending and left anterior descending (LAD) coronary arteries. Maleki and Lange[314] described two cases of acute MI in young oral contraceptive users. On angiography, a segmental occlusion of the LAD coronary artery was apparent in one case, but in the other the only detectable abnormality was some slight irregularity in the proximal LAD coronary artery. The authors proposed several explanations for this, including thrombotic occlusion of the coronary microvasculature, a local arteritis with complete regression and healing, coronary embolisation with complete lysis, local thrombus formation with regression and healing, or severe coronary spasm. In contrast, coronary spasm was proposed as an explanation for the findings reported by Jugdutt et al.[315] These authors reviewed the literature up to 1983 on angiographic and autopsy studies of oral contraceptive users who had experienced a MI and noted that, of the 21 women described, 15 showed no angiographic evidence of coronary obstruction and 5 showed no such evidence on autopsy. The authors also noted that the only identifiable risk factor apart from oral contraceptive use in these women was cigarette smoking. In the two cases they described, a coronary constrictor response to ergometrine was apparent and this was relieved by nitroglycerin. Both women were cigarette smokers and the authors suggested that vasospasm resulting from a synergistic effect of oral contraceptive use and cigarette smoking on platelet aggregation and vasoconstrictor release might account for the infarctions in these women.

In reviewing reports of 110 cases of MI with normal or near-normal coronary angiograms, Raizner and Chahine[208] found 9 out of 27 women to have been using oral contraceptives at the time of their MI; 67% of the oral contraceptive users were cigarette smokers compared with 42% in the group as a whole. Landau et al.[316] described a MI event in a 25-year-old woman who was a heavy smoker with a family history of coronary artery disease. Angiography a month after the event was normal

and ergometrine provocation did not induce coronary spasm. Waters and colleagues[279] evaluated coronary angiograms in 239 women aged less than 45 years; 112 had normal coronary arteries and 23 had stenoses of <50% lumen diameter. Hyperlipidaemia, hypertension, diabetes mellitus, cigarette smoking and a family history of coronary disease were significantly increased among the women with positive angiograms. Among the women with significant coronary disease, 37% had used oral contraceptives, whereas 27% of the women with normal angiograms had used oral contraceptives. This difference was not significant but, among those with segmental wall motion abnormalities, the oral contraceptive users had significantly more anterior segment abnormalities than the non-users (90 and 60%, respectively). In accord with other reports, most stenoses were found in the LAD coronary artery, but the authors noted that this was the case in young women in general who have experienced a MI, so the preponderance of such lesions in oral contraceptive users was not surprising.

The Studies of Engel and Colleagues

The series of reports from Engel and colleagues[317-320] represents the largest study of angiographic characteristics in oral contraceptive users who survived a MI. In their first report, angiographically-normal coronary arteries were found in 4 users of estrogen/progestogen combinations, who had survived a MI. Regional myocardial perfusion, studied in 3 of these patients, was found to be abnormal despite there being no apparent occlusion of the coronary arteries. This would imply structural changes in the coronary vasculature at the pre-capillary or capillary level. The infarction itself might then have been caused by coronary artery spasm or thrombosis with subsequent recovery of normal vessel patency. The most inclusive of the reports from Engel[320] involved coronary angiographic studies in 173 women who had experienced a MI before 50 years of age. Angiograms were classified as being atypical or typical of patients with coronary atherosclerosis. Atypical angiograms were defined as those showing no lumenal irregularity, one isolated lumenal irregularity, or one isolated smooth focal lesion of more than 50% luminal diameter. 47 women had taken oral contraceptives and had atypical coronary angiography, 31 had taken oral contraceptives and had angiographic evidence of coronary atherosclerosis, 18 had not taken oral contraceptives and had atypical coronary angiography, and 77 had not taken oral contraceptives and had angiographic evidence of coronary atherosclerosis. Therefore, among non-users who had experienced a MI, 19% of women had atypical coronary angiography, whereas among the oral contraceptive users the equivalent figure was 60% (fig. 14). This preponderance of atypical angiographic profiles in oral contraceptive users accords with earlier case reports. The distribution of coronary artery obstructions was similar in all 4 groups, however, with the LAD artery being the most frequently involved vessel, followed by the right and the left circumflex coronary arteries. In common with previous authors, Engel noted that among the oral contraceptive users with atypical angiography there was a relatively low prevalence of atherogenic risk factors, such as hypercholester-

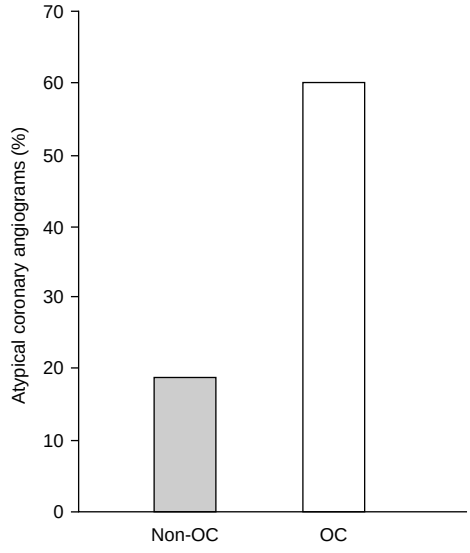

Fig. 14. Percentage of atypical coronary angiograms in young women who have survived a myocardial infarction, according to whether they were using oral contraceptives (OC).[320]

olaemia, hypertension, family history and glucose intolerance (average number of risk factors 0.4). Cigarette smoking was the one exception, with 64% of the oral contraceptive users with atypical angiography being smokers. Among the non-users with atypical angiography, 39% of the women were smokers (average number of risk factors 1.0). Previous angina pectoris was rare in these groups (<11%). Oral contraceptive users and non-users with angiographic evidence of coronary atherosclerosis did not differ, either in average number of risk factors or prevalence of cigarette smoking (fig. 15). This study[320] could not determine whether oral contraceptive use contributed to the emergence or progression of atherosclerosis in those women with abnormal coronary angiography, but did provide strong evidence for an atypical angiographic profile in the majority of oral contraceptive users who had experienced a MI. The smooth focal obstructions seen in the coronary angiograms in the oral contraceptive users are consistent with platelet or thrombus accumulation, and the normal coronary arteriograms suggest that occlusion was due to coronary vasospasm or that there was rapid thrombolysis. It is noteworthy that in two of the oral contraceptive users single focal lesions causing 75% obstruction were seen, but on re-angiography at 5 and 23 months these had regressed to 'mere luminal irregularities'. The smooth, rounded appearance of isolated segmental coronary lesions has been noted previously.[233]

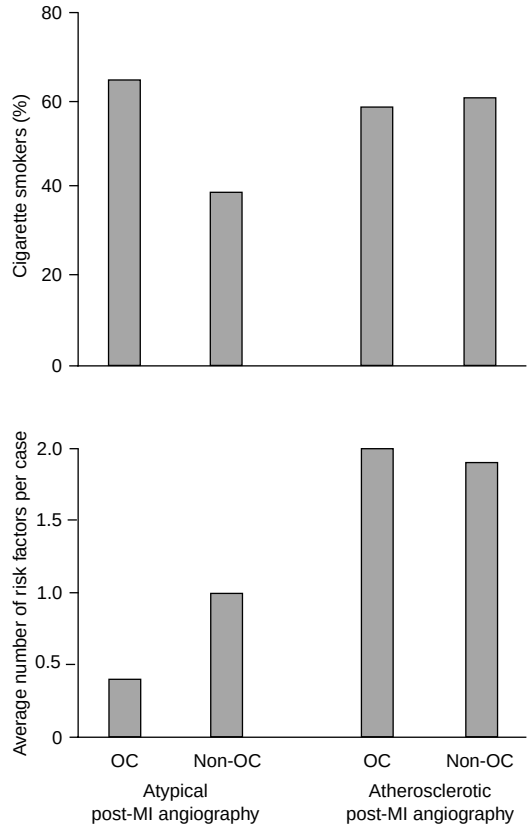

Fig. 15. Average numbers of risk factors and prevalence of cigarette smoking in young women who have survived a myocardial infarction (MI) according to oral contraceptive (OC) usage and coronary angiography.[320]

Autopsy Studies

Death from vascular causes in oral contraceptive users is largely due to occlusion of the coronary arteries or the cerebral, pulmonary, mesenteric or hepatic vessels. In the few autopsy studies that have been reported in oral contraceptive users dying from these conditions, thrombotic occlusion of the vessels concerned has been found. It is difficult to assess the extent to which these thrombi formed before or after death. It should also be kept in mind that consideration of case reports may give an exaggerated impression of the frequency of atypical cases, since it is these that are most likely to be considered worth reporting.

With regard to the coronary arteries, in 1965 Hartveit[104] described a single case in which a 32-year-old woman who had been taking oral contraceptives for 5 months was found on postmortem to have normal large coronary vessels, but small vessels plugged with thrombi. It should be noted, however, that a subsequent examination of the evidence relating to this case indicated that the case was more likely to have been one of chronic myocarditis with accompanying coronary arteritis than MI.[321] In the same year, Naysmith[105] described a similar case in whom a segmental occlusion by thrombus of the left anterior descending coronary artery was found on autopsy, and Osborn[106] re-

ported occlusion in 3 of the coronary arteries in a woman, subsequently found to have been an oral contraceptive user, who had died of a MI. He noted that it was very rare to find thrombus in more than one of the main coronary trunks, and it was this that had led him to suppose that oral contraceptive use had been involved. In 1969 Stout[322] described a woman who had experienced a nonfatal MI at 32 years of age followed by a fatal event at age 37. After her first MI, she was found to have moderate hypercholesterolaemia but haemostatic parameters within the normal range (measurements included fibrinogen, fibrinolytic activity, prothrombin time, silicone coagulation time and thrombin generation). She was taking estrogen replacement therapy at the time of both events, having undergone oophorectomy at age 29 years. Autopsy revealed occlusion of the right coronary artery by recently formed red thrombus. Underlying this was an accumulation of loose connective tissue overlying the endothelium. Platelet thrombi were attached to this connective tissue, completely blocking the lumen at one location. Subendothelial clusters of foam cells were present, but there was no atheroma or signs of inflammatory change. Another case report also noted thrombosis of the anterior descending branch of the left coronary artery in the absence of detectable atherosclerosis.[323] Further support for the prevalence of thrombosis as a mechanism for coronary occlusion came from the mortality study of Mann and Inman.[78] Among the 104 deaths investigated postmortem, 83% of the oral contraceptive users had evidence of coronary thrombus, in contrast to 55% of the non-users.

Autopsy studies of oral contraceptive users dying of stroke have generally revealed thrombotic occlusion of the superior sagittal sinus and cerebral veins, with occasional reports of thrombotic occlusion of the cerebral arteries.[324-327] Heyman et al.[145] compared the features of cerebral artery occlusion in women taking and not taking oral contraceptives. They noted a relative lack of occlusive lesions in extracranial arteries in oral contraceptive users, in contrast to the pattern seen in association with atherosclerotic cerebrovascular disease.

A number of autopsy reports, from 1963 onwards, have described cases of thrombus in the mesenteric vein in oral contraceptive users,[328-335] as well as in the mesenteric artery.[329,332,333,336-339]

Histopathology

In addition to autopsy studies identifying venous and arterial sites of thrombotic occlusion in oral contraceptive users, some studies have examined the histopathology of the vessel wall at these sites. Although reports are scarce, they are remarkably consistent in identifying distinct proliferative changes in the vessel wall of oral contraceptive users. These lesions appear to occur independently of thrombus formation, but they may nevertheless act as a focus for thrombotic, or even proliferative, occlusion of the vessel lumen.

In 1970, Irey and coworkers[340] reviewed samples of carotid, coronary, mesenteric and pulmonary arteries, and mesenteric and portal veins from 20 cases of fatal thromboembolism associated with the use of oral contraceptives. The material was from a tissue registry and had been collected between 1966 and 1968. Control material comprised samples from 22 women with fatal thromboembolism who had not been taking oral contraceptives. None of the oral contraceptive users had any known conditions that would have predisposed to thrombosis. Women had been using oral contraceptives for between 1 to 13 months prior to death. Overall, thrombi and associated lesions of the vascular wall were not localised to any specific area of the vasculature, although thrombi were limited to the pulmonary artery in 9 patients. Three-layered thrombi with underlying histochemical changes in the vessel wall were found in 19 cases. In 4 cases, endothelial proliferation and intimal thickening alone were found, and in 1 there was focal nodular fibrous thickening of the intima, media and adventitia without endothelial proliferation. Where thrombus was detected, the intima tended to be thickened beneath the thrombus by fibrous tissue, and the media showed thickening or thinning, with fibrosis and disruption of the muscular, elastic and fibrous components. On the basis of their structural features, these thrombi were estimated to be at least a

week old. There was no sign of underlying atherosclerosis and only one instance of an inflammatory reaction. Those lesions characterised by endothelial and intimal proliferation were found in the smaller pulmonary vessels. Broad papillary projections from these lesions almost filled the vessel lumen in some instances. Among the 22 controls, only one had lesions resembling those seen in the oral contraceptive users but she subsequently proved to have been taking oral contraceptives for 6 weeks prior to her death. Irey et al. concluded that in many instances the occluding thrombi had formed locally rather than from embolisation and that the lesions developed over days or weeks. The finding of vascular lesions without occlusive thrombi strongly suggested that changes in the vessel wall were primary. In a further study, Irey and Norris[341] confirmed these findings and extended their observations to include material from pregnant and postpartum women.[341] Lesions similar to those seen in oral contraceptive users were found in these women and the authors concluded that high plasma concentrations of reproductive steroids could cause vascular compromise by inducing intimal proliferation. This latter possibility was in accord with the observation by Manolo-Estrella and Barker[342] that in pregnancy the aorta undergoes changes which include hypertrophy and hyperplasia of intimal smooth muscle cells. In a later report, Irey et al.[343] addressed the issue of vascular changes in stroke patients who had been using oral contraceptives. Three cases were examined and each showed the characteristic intimal hyperplasia that had been identified in the earlier studies present in the arteries supplying the brain. Overlying thrombus was seen with some but not all of these lesions. These vascular changes were seen in only 2 of 40 other women aged 20 to 40 years who had died suddenly of nonvascular causes, and in 9 of 67 women aged 19 to 39 years who died of a variety of cardiovascular causes.

Intimal thickening and endothelial proliferation in the vasculature of oral contraceptive users have been described in a number of other reports of postmortem histopathology.[324,326,327,339,344-346] In none of these studies were there any signs of atherosclerosis in the affected vessels. Endothelial proliferation was clearly apparent in several reports. For example, Altshuler and colleagues[324] found cerebral and myocardial microvascular endothelial proliferation with severe lumenal narrowing associated with a fatal cerebrovascular accident in an oral contraceptive user. Poltera,[327] in his account of histopathologic changes in the cerebral vasculature of two women who had died of stroke, described substantial endothelial hyperplasia in the lateral lacunae of the dura mater, with thrombus formation at the margins of these regions. Heavy cigarette smoking was noted in several of these cases. For example, Lamy and colleagues[339] observed vascular lesions, like those described by Irey and colleagues,[340] in the celiac and mesenteric arteries of a young woman who had died of a massive intestinal infarction while taking a medium estrogen dose oral contraceptive combination. The woman was a regular cigarette smoker (25 to 35 per day) and the authors noted that in the two other reports of visceral artery thrombosis which had included details of cigarette smoking, the cases had been heavy cigarette smokers.[332,338]

Evidence for an impact of oral contraceptives on the vascular wall was the subject of an important review, published in 1980 by Basdevant et al.[196] They concluded that the most consistent lesion was a fibromuscular thickening of the intima, with other lesions involving medial or endothelial proliferation. As with the fibromuscular changes, the changes in the endothelium could occur in isolation or in combination with other types of change. Endothelial lesions tended to predominate in veins, but both veins and arteries could be similarly affected in a single subject. Case studies describing atherosclerotic changes in oral contraceptive users who had died as a result of vascular accident were the exception. The authors addressed 3 major questions with regard to the vascular lesions found in oral contraceptive users: (i) were these lesions specific to oral contraceptive users? (ii) were they a primary effect of the contraceptive steroids or secondary to humoral changes? and, (iii) to what ex-

tent were they responsible for the clinical sequelae? They concluded that the estrogen component of the combined oral contraceptive was primarily responsible, although the effect might be modified by the progestogen. Whether the effect was specific to alkylated estrogens was unclear but it was noted that similar lesions were seen in pregnancy, suggesting an effect of high estrogenic activity rather than estrogen type. The mitogenic effect of estrogens and the presence of estrogen receptors on vascular endothelial cells was invoked to support a direct effect of estrogens in the induction of intimal and endothelial hyperplasia; the direct involvement of these lesions in thrombogenesis was evident in the autopsy studies. Intimal and endothelial hyperplasia could in some cases cause complete occlusion of the vessel lumen. Non-atheromatous arterial stenosis could also result from the fibromuscular proliferative changes seen in the media of some oral contraceptive users. It was also noted that combined oral contraceptives could weaken the muscular and connective tissue of the arterial wall (see section 4.1) and so promote the formation of dissecting aneurysms.

Histopathologic studies on postmortem material suggest that intimal and endothelial hyperplasia is a rare phenomenon, representing an idiosyncratic reaction to oral contraceptive steroids in certain susceptible women.[347] However, there are some reports, albeit mainly concerning reproductive tissues, that indicate that such lesions can be found in apparently healthy women taking oral contraceptives.[348-350] For example, Blaustein and colleagues[348] and Grant[349] found endothelial and intimal smooth muscle cell hyperplasia in the vessels of the endometrium, skin, colon, cervix, breasts and ovaries of women taking oral contraceptives. The majority of these studies on healthy women concerned reproductive tissue, which might be expected to be particularly sensitive to gonadal steroids. However, studies by Schenker et al.,[351] using dye injection of the vasculature of the retina in women taking oral contraceptives revealed narrowing of the retinal vessels, and further evidence for a direct adverse effect of oral contraceptive steroids on the vascular wall comes from the RCGP study in which disorders of the capillary microcirculation were linked to oral contraceptive use.[20]

These histologic studies strongly suggest that oral contraceptives may induce focal intimal hyperplasia, involving smooth muscle cell proliferation and increased collagen and mucopolysaccharide deposition, sometimes with endothelial proliferation. As Irey et al.[343] pointed out, such intimal proliferation may be seen in various of disease conditions and is by no means unique to oral contraceptive users. However, oral contraceptive therapy was the only distinguishing feature in the cases presented. This and evidence from animal studies reviewed in section 3.2.2 suggests that the lesions were steroid-induced.

3.2.2 Studies in Animals

The observation of intimal hyperplasia in women taking oral contraceptive steroids was anticipated by animal studies. Proliferation of the cells lining vascular spaces in estrogen-induced experimental fibroids was described by Lipschutz[352] in 1930 and similar changes in the vasculature of animals given estrogens was described by Bruzzone[353] in 1948. These observations were replicated in a number of subsequent studies, both of the effects of exogenously administered estrogens and of changes occurring during pregnancy.[354-361]

Danforth and colleagues[354] examined aortae from rabbits given contraceptive steroids (mestranol/noretynodrel, 0.2 mg/day intramuscular injection) and found smooth muscle cell hypertrophy and hyperplasia. In the study of Gammal,[360] the effects of ethinylestradiol and the progestogen chlormadinone on aorta and carotid, mesenteric and renal arteries of the rat were studied. Ethinylestradiol promoted intimal thickening and there was evidence that, in the long term (>30 days), the progestogen could oppose this effect. In a further study, by Gammal and Monture,[362] significant aortic intimal thickening was confirmed in ovariectomised female rats given ethinylestradiol alone for 10 days. In several of these studies, changes in vascular structure and permeability were found, in addition to the histological changes outlined here.

These findings are described below (section 4.1) in relation to potential mechanisms that could contribute to these histological observations.

The potential importance of these studies of vascular effects of oral contraceptive steroids in animals appears to have been almost entirely obscured by the interest that developed during the 1980s in the effects of female sex steroids in animal models of atherosclerosis. Evidence summarised above indicates that these may be of only marginal relevance to the general problem of vascular disease in oral contraceptive users (although they may be relevant to the effects of oral contraceptive steroids in women already at risk of developing atherosclerosis, or who already have established atherosclerosis). In models involving the fat-fed cynomolgous monkey, contraceptive estrogens protected against atherosclerosis,[363,364] but this was only seen in subdominant females.[365] These animals have low plasma estrogen levels so this protection becomes one aspect of the potential that estrogen replacement has for opposing the increased risk of atherosclerosis associated with estrogen deficiency. Nevertheless, it is of some interest, in relation to the adverse arterial effects of the oral contraceptive progestogen seen in some epidemiological studies, that the protective effect of ethinylestradiol alone in this model is diminished in the presence of a progestogen.[366]

3.3 Conclusions

The studies of vascular pathology in oral contraceptive users described in section 3.2.1, and the corollary evidence from animal studies, indicates that oral contraceptive steroids can promote distinctive proliferations of intimal and endothelial cells and that these changes can be associated with thrombotic occlusion. Such changes are not typical of thrombosis in general. Furthermore, in oral contraceptive users who have experienced a MI, there is angiography that is not typical of atherosclerotic disease and this is associated with a risk factor profile that is also atypical. Angiography *per se* cannot exclude the presence of atherosclerosis, since it does not appear to detect a substantial proportion of atherosclerotic lesions. However, the distinctive risk factor profile among those who have experienced a MI but who have a normal coronary angiogram argues in favour of other processes predominating. These observations have been taken to indicate a thrombotic rather than atherosclerotic basis for coronary artery occlusion. This is not a valid distinction, however, since coronary occlusion associated with atherosclerosis is, in any case, generally thrombotic. A more appropriate distinction would be between non-atherosclerotic endothelial damage and dysfunction and atherosclerotic plaque rupture as underlying causes of coronary artery thrombosis.

The confounding influence on oral contraceptive studies of the perceived importance of atherosclerosis in arterial disease should be noted, since this has been particularly influential in attempts to use animal models of occlusive vascular disease to evaluate the effects of oral contraceptives. These models have largely depended for their conclusions on evaluation of the influence of contraceptive steroids on the development and progression of atherosclerotic lesions induced by dietary manipulations. Such work has highlighted the beneficial effects of estrogens on the atherosclerotic process as such, but has left untouched the potential of contraceptive steroids to influence vascular structure, endothelial function, thrombotic tendency and factors that can affect these different aspects of the vasculature. Possible mechanisms that might contribute to the distinctive features of vascular occlusion in oral contraceptive users are considered in section 4.

4. Mechanisms of Occlusive Vascular Disease in Oral Contraceptive Users

Possible mechanisms that might account for occlusive vascular disease in oral contraceptive users may include direct effects of contraceptive steroids on the vascular wall, oral contraceptive-induced changes in factors that might promote endothelial dysfunction, and oral contraceptive-induced changes in factors that might promote thrombosis. The appearance of intimal and endothelial hyperplasia in

areas of vascular occlusion, and the importance of cigarette smoking and hypertension in arterial disease in oral contraceptive users directs attention to factors which act on vascular integrity and endothelial function. On the other hand, the consistently increased risk of VTE, the increased risk of thrombotic stroke and the profile of resolving coronary thrombus directs attention to changes in factors of the haemostatic system.

The various measures to be considered here have generally been discussed as risk factors for atherosclerosis or thrombosis. In the present work, however, they are being considered primarily as factors that are affected by oral contraceptive steroids and that might explain the particular epidemiological and pathological characteristics of occlusive vascular disease in oral contraceptive users. Selective consideration of more general aspects of risk factor theory is worthwhile, however, since there are some issues that are of particular importance in interpreting the significance of oral contraceptive-induced changes.

4.1 Risk Factors

4.1.1 Risk Factors and Proximal Causation

In its original and still most common usage, the term 'risk factor' denotes any observable characteristic that confers on an individual significant risk of occlusive vascular disease. Thus, male gender, cigarette smoking, high serum cholesterol levels or use of high estrogen dose oral contraceptives might all be considered risk factors. In one survey, 272 such 'risk factors' for coronary artery disease were identified.[367] It is important to bear in mind, however, the proximate level of cause that is being considered. Thus cigarette smoking is a risk factor, but so too are the increased fibrinogen levels and free radical generation induced by cigarette smoking. In fact, cigarette smoking may act as a risk factor by adversely affecting these other factors, which are themselves directly responsible for vascular damage and occlusion. This is a critical distinction that must be made in any discussion of cardiovascular risk. In an early analysis of data from the Framingham Study,[368] it was concluded that postmenopausal estrogen use did not protect against coronary heart disease because, when HDL cholesterol was included in the multivariate analysis, postmenopausal estrogen use ceased to be a significant predictor of reduced coronary heart disease. The apparent protective effect of postmenopausal estrogens was therefore 'explained' by the protective effect of high HDL levels. But postmenopausal estrogens increase HDL levels, and it is precisely this increase in HDL that could be mediating their protective effect. The Framingham multivariate analysis, in fact, strengthens the evidence in favour of a protective effect of postmenopausal estrogens by suggesting a possible mechanism through which they might act.

4.1.2 Establishing a Risk Factor

To fully qualify for the status of risk factor, a potential risk factor must have been tested in a series of increasingly rigorous investigations. In the first instance, it must of course be identified; levels must be shown to be significantly altered in those with the disease compared to healthy individuals. Levels of the factor must then be shown to predict the subsequent development of disease in epidemiological studies, and studies at the biochemical or physiological levels must suggest mechanisms by which the factor might be involved in pathogenesis. Finally, if all these preliminary investigations still provide strong support for the importance of the factor in question, it must be shown that manipulation of levels of the factor induce the expected changes in disease onset or severity. This may be carried out in animal models of the disease, employing dietary intervention, infusion or transgenic manipulation, and in humans by dietary, pharmacological or other intervention, providing the means are available to do so safely. Only two potential risk factors have achieved full risk factor status according to this scheme: hypertension and high LDL cholesterol levels. The latter now provides the classic model of a risk factor. It is noteworthy that 45 years elapsed from the first widely publicised proposals that cholesterol might be an important factor in the development of coronary heart disease, to the demonstration that pharmaco-

logical inhibition of cholesterol synthesis in a disease-free population could significantly lower the subsequent incidence of coronary heart disease. It is unlikely that the many other potential risk factors that have been identified will all be investigated with similar rigour. As described in this section, HDL cholesterol and triglycerides are in an advanced stage of investigation as risk factors, but other potential factors are at a considerably earlier stage of development. Nevertheless, a potential risk factor should not be disregarded merely because it has not passed every qualifying hurdle. Rather, its status in the developing sequence of investigations should be carefully identified and the importance given to changes in the factor modified accordingly. The very long term strategy needed to establish a risk factor should also be born in mind. Failure to recognise this can lead to frustration and uncritical disregard of potentially useful data. It can also lead one to forget that the ultimate purpose of risk factor evaluation is to anticipate serious clinical events before they develop and thus, to take steps to prevent them ever happening. At the heart of risk factor evaluation is the potential for prevention.

4.1.3 Risk Factors Quantification

Supposing that an x% increase in the concentration of a given risk factor increases risk of a given disease by y%. It is then relatively straightforward to establish what mean percentage change in concentration of that risk factor is induced by use of a particular oral contraceptive formulation in a defined group of women, and to infer a commensurate change in disease risk. Unfortunately, this is, for the most part, an idealised example in that it assumes a continuous linear relationship between risk factor concentration and disease risk. In the few instances for which we do have reliable data, the relationship is rarely linear, and disease risk may increase sharply above a certain threshold. Overall changes in mean or median values then become relatively poor indicators of change in risk, and one must instead obtain some estimate of the prevalence of the risk factor levels which are associated with a significant increase in disease risk. To obtain a realistic estimate may be difficult. First, it may be misleading to refer to risk factor levels established at another centre in a different population, by a different group of investigators. Inter-laboratory standardisation may be nonexistent for many risk factors, so measures described in one report cannot be automatically applied to other studies. It may, nevertheless, be possible to set up a degree of internal standardisation in which some upper limit is defined according to, say, the 90th percentile cut-off for a particular risk factor distribution in a large group of healthy controls. The proportion of oral contraceptive users with values above this limit may then be determined, it being assumed that changes in the prevalence of values below this limit are of little clinical significance. Such an approach necessitates large sample sizes and a risk factor with a moderately broad numerical distribution. One must also be careful to relate such internal references to the available epidemiological information; for example, how comparable was one's reference group to the group of individuals in whom the quantitative link between disease risk and risk factor level was established? And at exactly what percentile limit did disease risk increase?

4.1.4 Risk Factor Interrelationships

The above considerations relate to the evaluation of a single risk factor. A complication in this scheme is that risk factor disturbances rarely occur in isolation. Rather there may be correlated changes in a range of risk factors which either augment the state of risk, as in the metabolic or insulin resistance syndrome,[369] or which tend to be mutually compensatory, as in some oral contraceptive-induced changes in the coagulation and fibrinolytic systems (see section 4.9). It then becomes unrealistic to consider potential risk factors in isolation. Evaluation of the impact of correlated risk factors on cardiovascular risk is still in its infancy. The problem centres on the fact that the classic multiple logistic regression techniques used to assess the predictive power of a particular risk factor are designed to identify independent risk factors, and they tend to give weight to those measures with the

least variation, be it analytic, intra-individual or inter-individual. Such measures are not necessarily more important in the disease process than factors with greater variance; it might even be argued that the latter are likely to be more important since an individual is more likely to be exposed at some point to extremes in the range of values encountered. One possible approach to the problem is to identify and quantify syndromes of risk factor disturbance. The statistical technique of factor analysis has the potential both for identifying significant clusters of risk factors and assigning a quantitative value to such clustering in each individual.[370,371] Such 'factor scores' are now being used as variables in prospective analysis.[372]

This section on factors which might influence occlusive vascular disease in oral contraceptive users is structured as follows: in the first part, possible direct effects of oral contraceptive steroids on vascular structure are considered; the next 6 parts are concerned with factors that have been considered primarily in relation to atherosclerosis, but which, in the context of the present work, may be more usefully assessed in relation to the endothelial damage and dysfunction that can, ultimately, lead to atherosclerosis; in the final part, factors of the haemostatic system are considered. It should be emphasised that these are not rigid distinctions. For example: HDL has manifold actions that promote endothelial health but can also act to inhibit smooth muscle cell proliferation and promote the antithrombotic effects of protein C; postprandial lipoprotein remnants can be taken up into the subendothelial space and contribute to endothelial damage but can also provide a surface for the activation of factor VII (FVII); insulin can stimulate smooth muscle cell proliferation and the synthesis of the antifibrinolytic factor, plasminogen activator inhibitor-1 (PAI-1); and homocysteine can promote oxidative damage of the endothelium, but also stimulate activation of factors X and V and inhibit expression of thrombomodulin and activation of protein C. More generally the workings of the vascular endothelium and the haemostatic system are closely interrelated, and this may underlie the commonality of many of these clusters of effects, which tend to work together to either promote or inhibit vascular occlusion.

4.2 Direct Effects on the Vasculature

Direct effects of oral contraceptive steroids on the vasculature that might underlie the distinctive vascular pathology in oral contraceptive users described above (section 3) and consequent vascular occlusion have, necessarily, been mainly the subject of animal studies. As previously mentioned (section 3.2.2), histological observations in animals of intimal and endothelial hyperplasia in response to contraceptive steroids have been accompanied by further investigation of the underlying structural changes that might contribute to these proliferative responses and these findings are summarised here.

Danforth and colleagues[354] examined aortae from rabbits given contraceptive steroids (mestranol/noretynodrel, 0.2mg/day intramuscular injection). They found loss of the normal elastic configuration of the aorta, decreased acid mucopolysaccharide and fragmentation of reticulum fibres in stained section. These changes were reversed when steroid administration ceased.[373] The partial breakdown of the arterial elastic lamina seen in these studies was also apparent in the investigations of Albert[356] and Wexler.[359] Wexler's studies of uterine vein morphology in the pregnant rat showed that the vessels had become thin walled, flaccid and greatly dilated. Gamma and Monture[362] examined the permeability of aortic endothelium in ovariectomised female rats given ethinylestradiol for 10 days. These animals showed significant intimal thickening after treatment with ethinylestradiol, associated with an increase in the uptake of albumin-bound dye across the endothelium into the aortic wall. A similar finding was reported by Almén et al.[374] with respect to endothelial permeability to silver ions in estrogen-treated rats.

These studies in animals suggest a breakdown in vessel wall structure and an increase in endothelial permeability in response to estrogen and, in accord with this, there are a few reports of increased venous

distensibility and reduced blood flow in women exposed to high sex steroid concentrations in the course of the menstrual cycle, pregnancy or oral contraceptive use.[375,376] Furthermore, increased capillary permeability to proteins in oral contraceptive users has been reported,[377] an effect which was inferred in earlier studies of insulin metabolism.[378]

A possible explanation for this general pattern of disruption of the vascular elastic matrix, loss of vessel tone and increased endothelial permeability may be found in the observation by Wingrove et al.[379] of an estrogen-induced dose-dependent increase in the expression of matrix metalloproteinases (MMPs) secreted by cultured vascular smooth muscle cells. These highly active enzymes break down the collagen and elastin of the intercellular matrix of the vascular intima. They are susceptible to tissue inhibitors of matrix metalloproteinases (TIMPs) and there is preliminary evidence that estrogens may increase MMP activity by decreasing the expression of TIMPs (personal communication; C. Wingrove, 2000).

With regard to the proliferative lesions consistently seen in humans and animals given contraceptive steroids, increased endothelial permeability would be expected to result in increased exposure of subendothelial cells to growth-promoting substances which could then stimulate abnormal hyperplasia in the endothelium and intima. There might also be oral contraceptive induced increases in such growth-promoting substances. Bagdade and Subbaiah[380] observed that serum from oral contraceptive users stimulates the proliferation of arterial smooth muscle cells *in vitro*. This activity was heat stable, nondialysable and contained in the lipoprotein-deficient serum fraction. Addition of oral contraceptive steroids did not enhance the mitogenicity of control serum, indicating that the active component was a metabolite of the steroids or another growth-promoting substance whose synthesis had been stimulated *in vivo*. Candidates included mitogens released from platelets during coagulation – platelet concentrations possibly being greater in the oral contraceptive users – or increased growth hormone levels that were found in the oral contraceptive users. A subsequent study compared fasting serum levels of the arterial smooth muscle cell mitogens growth hormone, insulin, insulin-like growth factor (IGF)-I and -II, platelet-derived growth factor, β-thromboglobulin, and platelet factor 4 in oral contraceptive users and non-user controls.[381] There were no significant differences, although numbers of participants were relatively small. An increase in corticosteroid activity in response to oral contraceptive estrogens[382] might also be relevant, since intimal hyperplasia in coronary and visceral arteries has been observed in spawning salmon in association with increased plasma levels of 17-hydroxycorticosteroids.[383]

The observations described here are limited, but are, nevertheless, consistent with there being an estrogen-induced increase in MMP activity, leading to disruption of the vascular matrix, loss of vessel tone and increased endothelial permeability. Increased endothelial permeability then leads to exposure of the subendothelial space to growth promoting substances, which may themselves be circulating in increased levels in response to contraceptive steroids. These then induce the proliferative lesions observed in studies of vascular pathology in oral contraceptive users.

Loss of venous tone, and the accompanying tendency to venous stasis, would be expected to increase risks of venous thrombosis. Moreover, the proliferative lesions seen in association with oral contraceptive use are likely to be accompanied by disruption of blood flow and by endothelial dysfunction. Lamy et al.[339] considered that '. . . endothelial alterations produced by the intimal hyperplasia are highly thrombogenic and lead to the formation of a thrombus overlying the altered endothelium'. The complex of changes in vessel wall structure induced by estrogens might then be an important factor in thrombotic occlusion, since breakdown of vessel structure and loss of vessel tone, with increased permeability and the emergence of proliferative lesions might be expected to occur together in localised areas. Such a complex of changes focuses attention on their role in venous

thrombosis, but arterial occlusion might also be promoted by the accompanying endothelial dysfunction.

However, it should be emphasised that this is a very under-researched area. Existing evidence is highly suggestive and indicates that the estrogen component of the oral contraceptive is important, but there has been no rigorous programme of research to establish just how much such changes matter in occlusive vascular disease in oral contraceptive users. Moreover, nothing is known of the effects of estrogen dose in this area. Neither is it known whether progestogens modify the effects of estrogens, or whether other risk factors, for example cigarette smoking or hypertension, play a role.

4.3 Lipid and Lipoprotein Metabolism

Disturbances in plasma lipid and lipoprotein metabolism are strongly linked to the development of atherosclerosis. However, the importance ascribed to atherosclerosis as a mechanism for vascular occlusion has tended to hinder resolution of controversies over the relevance of oral contraceptive-induced changes in lipid and lipoprotein metabolism. In addition to a role in plaque development, lipids and lipoproteins can influence many other aspects of vascular disturbance and damage, in particular those relating to the haemostatic system and endothelial function. Thus oral contraceptive-induced changes in lipid and lipoprotein metabolism may be relevant both to thrombosis and endothelial dysfunction in oral contraceptive users.

The metabolism of cholesterol, triglycerides and other lipids is subject to various genetic and environmental influences, which are mediated in part by endogenous hormones, including estrogens. Exogenously administered hormones, including those in combined oral contraceptives, can be even more effective in altering plasma lipoprotein metabolism. Steroids administered orally may be particularly potent, not only on account of modifications to their structure but also to the high steroid concentrations that the liver is exposed to during oral administration.

Associations between changes in plasma lipoprotein metabolism and arterial disease were identified over 30 years ago, and interest in changes in lipoprotein metabolism in oral contraceptive users has evolved out of their implications for increased risk of arterial disease in oral contraceptive users. In the absence of adequate epidemiological information, changes in lipoprotein metabolism have been used as surrogate indices of arterial disease risk. This has been particularly important with regard to newly introduced formulations for which epidemiological information is often absent. Use of lipid and lipoprotein levels as surrogate indices is controversial, partly because of our inadequate understanding of the pathology of arterial occlusion in oral contraceptive users.

Such controversy reflects the importance that has been ascribed to the role of lipoprotein metabolism in arterial disease risk, and although oral contraceptives affect other metabolic and physiological systems linked to cardiovascular risk, lipoprotein metabolism is the single most investigated metabolic aspect of oral contraceptive safety. Indeed such investigations have in part driven oral contraceptive reformulation. Formulations containing desogestrel, gestodene or norgestimate have been promoted on the basis of their ability to raise HDL levels.

4.3.1 Plasma Lipoprotein Metabolism and Risk of Occlusive Vascular Disease

Lipids include cholesterol, triglycerides, nonesterified fatty acids (NEFA) and phospholipids. They act as a major source of energy, provide an essential component of cell membranes and, in the case of cholesterol, act as a precursor for steroid hormone synthesis. Because of the relative lack of polar elements in their structure, lipids have very limited solubility in water. To be transported within body fluids, they are therefore encapsulated in the nonpolar regions of proteins or protein complexes. In the case of NEFA, the protein vehicle is albumin, whereas other lipids are complexed with one or more specific proteins (apolipoproteins) to form macromolecular complexes termed lipoproteins. The structural integrity of plasma lipoproteins, and

their ability to act as vehicles in body fluids, is further enhanced by the ability of relatively polar lipids, such as phospholipids and free cholesterol, to form a surface 'coat' which can stabilise the hydrophobic 'core' components, primarily cholesteryl esters and triglycerides.

Lipoproteins show considerable variation in structure and composition. Classification systems have generally been based on the physicochemical characteristics of these particles. The classification therefore depends on the methodology employed to separate different lipoprotein species. Size, electrophoretic mobility or density have all been employed, with the most widely used nomenclature being based on the density of these particles relative to water. This is dictated by the relative amounts of constituent lipids and proteins. In decreasing order of density, the 5 major classes are: (i) HDL, protein-rich lipoproteins originating in the liver and small intestine, which transport cholesterol from peripheral tissues to the liver for disposal, and which also contribute to endothelial health and the regulation of triglyceride metabolism; (ii) LDL, cholesterol-rich lipoproteins which deliver cholesterol to peripheral tissues following their appearance in the plasma predominantly as the partially-catabolised end-products of (iii) intermediate lipoproteins (IDL) or of (iv) very-low density lipoproteins (VLDL). These are triglyceride-rich lipoproteins of hepatic origin which distribute both dietary and endogenously-synthesised triglycerides to peripheral tissues. The least dense lipoprotein particles are (v) chylomicrons, very large triglyceride-rich particles of intestinal origin which, in normal individuals, are only present in the plasma in the hours following a fatty meal.

In accord with a role in cholesterol elimination, endothelial function and metabolic regulation, HDL levels are low in individuals at increased risk of MI and stroke. In contrast, LDL and the various triglyceride-rich lipoproteins are potentially atherogenic and levels of these lipoproteins are high in those at risk. Impaired catabolism of chylomicrons and other triglyceride-rich lipoproteins may increase arterial disease risk. A further factor in relationships between lipoprotein metabolism and arterial disease is an LDL-like particle, lipoprotein(a) [Lp(a)], which may have especially adverse effects on the arterial wall. It should also be noted that the quality of lipoproteins may be as important as their plasma levels. For example, oxidatively modified LDL appears to be particularly atherogenic. Plasma lipoprotein classes have been further distinguished into numerous subclasses, often in an attempt to obtain better predictive power. The extent of such heterogeneity depends on factors such as the specific analytical system used for their separation, but there is reasonable consensus as to at least 3 VLDL subclasses, 7 LDL subclasses and 7 HDL subclasses.

The atherogenic consequences of ingress of triglyceride-rich lipoproteins and LDL into the subendothelial space have been described previously, and the ability of oxidatively-modified LDL to directly impair endothelial function should also be re-emphasised. The antiatherogenic properties of HDL, first recognised from negative associations between HDL concentrations and risk of coronary heart disease, have been commonly ascribed to the ability of this lipoprotein to mediate reverse cholesterol transport, whereby cholesterol is abstracted from tissues and transported to the liver for excretion. Less familiar, but possibly of equal or greater importance, are the many other properties of HDL and its principal apoprotein, apoAI, which are continuing to be discovered and which, taken together, argue in favour of a major role for this lipoprotein in the promotion of effective endothelial function. The most recently described of these properties relates to enhancement of the anticoagulant properties of proteins C and S.[384] Other properties that have been described include membrane stabilisation and inhibition of the generation of procoagulant activity,[385] prostacyclin stabilisation,[386] inhibition of platelet aggregation,[387] inhibition of cytokine-induced expression of endothelial cell adhesion molecules,[388] inhibition of monocyte transmigration,[389] antioxidant activity[390] and inhibition of vasoconstriction.[391,392] It will be appreciated that many of these effects could

be mutually reinforcing, further emphasising the potential importance of HDL in endothelial health.

Different subclasses of a given lipoprotein class may differ in their effects on the arterial wall. For example, smaller VLDL and LDL particles may be more damaging than their larger counterparts. A reliable classification for HDL heterogeneity has proved elusive because of the extensive recycling and inter-conversion between different HDL subclasses (and other lipoproteins). With regard to the relative clinical importance of the different HDL subclasses, interest now focuses more on small lipid-poor particles displaying atypical (pre-β) electrophoretic mobility rather than larger HDL particles, such as HDL_2. These small 'acceptor' HDL particles, present in low levels in plasma but more prominent in interstitial fluid and lymph, may be of primary importance in some of the beneficial functions of this important class of lipoproteins.

In contrast to the major lipoprotein classes, there is only a limited amount of prospective data on the relationship between these subclasses and arterial disease. For example, although HDL_2 subclass levels are consistently low in survivors of MI (proportionately more so than total HDL levels), most prospective studies have not found levels of this subclass to be a strong predictor of subsequent vascular disease. Prospective epidemiological trials designed to evaluate the predictive power of specific cardiovascular risk factors are becoming increasingly difficult to perform, and the specialised techniques required for lipoprotein subclass estimation are rarely feasible in such a context.

Not only has this lack of clinical information obstructed efforts to ascribe clinical relevance to the changes induced by oral contraceptives, lipoprotein heterogeneity itself has proved problematic in studies in oral contraceptive users. Oral contraceptive-induced changes in the major classes of plasma lipoproteins may involve changes in subclasses which are of comparatively less potential relevance to arterial disease. For example, the increased triglyceride levels induced by oral contraceptives are due to increased plasma levels of larger VLDL, which may be less damaging to the endothelium than are smaller VLDL.

4.3.2 Oral Contraceptives and Lipoprotein Metabolism

Background

When oral contraceptives first came into widespread use in the early 1960s, existing evidence regarding the effects of sex steroids on lipoprotein metabolism and atherosclerosis would have supported a protective effect with regard to arterial disease. Such evidence included the gender difference in plasma lipoprotein levels (known to match the gender difference in MI risk); the menopausal conversion of the female plasma lipoprotein profile to that of the male, matching the higher risk of MI seen in postmenopausal women; and the inhibition of atherosclerosis when chickens and other animal models were treated with estrogens. Therefore, it is understandable that, given the more pressing issues at that time, Pincus[393] limited his reports on lipid metabolism in oral contraceptive users to one describing unchanged serum cholesterol and LDL levels in 41 women treated with mestranol 0.15mg plus noretynodrel 10mg for one year.

1960s: the Work of Aurell

The first formal report into the effects of oral contraceptives on lipoprotein metabolism was that of Aurell and colleagues[394] This was published in 1966, by which time use of oral contraceptives was widespread. Eight women treated with ethinylestradiol 0.05mg and norethisterone 4mg for one year were studied. Significant increases in serum total cholesterol and phospholipids levels (25 and 27%, respectively) were observed, but the most striking change was a 64% increase in triglyceride levels. HDL cholesterol levels fell by 24% and LDL cholesterol and protein levels increased by 21 and 36%, respectively. The authors were primarily concerned about the effect on LDL which they linked to the higher levels seen in male survivors of MI and in postmenopausal women.

1960s: Studies by Wynn and Colleagues

A more detailed evaluation of the effects of oral contraceptives on plasma lipid and lipoprotein lev-

els was that of Wynn et al.,[395] also published in 1966. Lipid and lipoprotein levels were compared between 102 women taking various oral contraceptive formulations (primarily mestranol 0.1mg plus etynodiol 0.5 to 2.0mg) and 75 non-users. Substantial elevations (70 to 80%) in fasting serum triglyceride levels were observed. Importantly, one third of the oral contraceptive users had triglyceride levels exceeding the highest level found in the non-users. Only minor increases in serum cholesterol levels were seen. This report also included data on lipoprotein classes distinguished by analytical ultracentrifugation. 43 of the women taking oral contraceptives were compared with 120 non-users. Levels of lipoproteins Svedberg units (S_f) 100 to 400 (S_f provide an index of the density of the particles from which a mass concentration may be derived) corresponding to large VLDL were 244% higher in oral contraceptive users, S_f 20 to 100, corresponding to small VLDL were 68% higher, S_f 12 to 20, corresponding to VLDL remnants or IDL were 13% higher, and S_f 0 to 12, corresponding to LDL were 24% higher. These findings are illustrated in figure 16. It is noteworthy that the modal density of the LDL fraction was 20% higher in oral contraceptive users than in controls, approaching the values typical of men. Although not measured directly, a fall in HDL levels was inferred since there was relatively little difference in total cholesterol levels between oral contraceptive users and controls, whereas VLDL and LDL levels were substantially increased. The convergence of the lipoprotein profile in oral contraceptive users towards the pattern seen in men was noted and the suggestion made that further studies of the clinical significance of these changes would be worthwhile. It was suggested that structural similarities between the progestogen and testosterone were the cause of these undesirable effects.

In contrast to the cross-sectional analysis employed in this first report, a subsequent investigation by Wynn et al.[396] was based on a longitudinal design. Essentially the same series of measurements were made and the conclusions of the earlier study were entirely confirmed. Changes in two

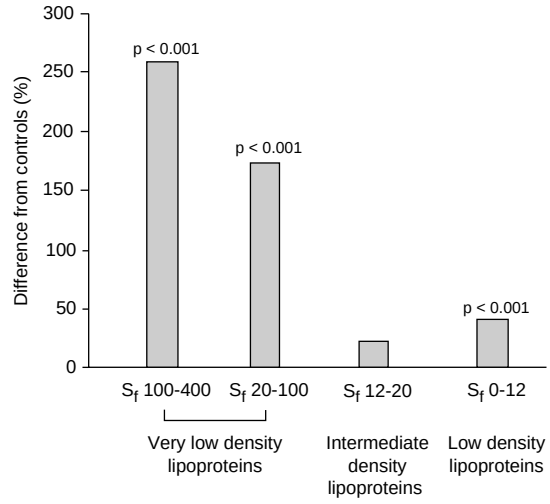

Fig. 16. Elevated lipoprotein levels in oral contraceptive users compared with non-users (controls), with separation by preparative ultracentrifugation. **S_f** = Svedberg units, an index of the density of a particle from which a mass concentration may be derived.[395]

groups were evaluated. These comprised 116 women studied before and while taking oral contraceptives, and 46 women studied while taking oral contraceptives and then after stopping therapy, the period off therapy being 2 months or more. The majority of oral contraceptive formulations being taken comprised mestranol 0.1mg combined with an estrane progestogen. The longitudinal design of this study enabled confirmation of the inference from the earlier report that the rise in triglycerides was ubiquitous rather than being confined to a few individuals; triglycerides increased in 111 out of the 115 women studied. Changes in both cholesterol and triglyceride levels were reversible. It was established that the increase in triglycerides was not due to the persistence of chylomicrons in the plasma. In searching for a mechanism for the marked increase in large VLDL and lesser increases in small VLDL and IDL, the authors speculated that the estrogen component of the combined oral contraceptive may be directly affecting lipoprotein metabolism, for instance, by increasing

hepatic synthesis of apolipoproteins, or may operate by inducing changes in endogenous hormones that might affect lipoprotein metabolism such as cortisol, thyroxine or growth hormone. Wynn's group pointed out that even small changes in plasma levels of a lipoprotein such as LDL might confer an appreciable attributable risk of arterial disease, given the large numbers of oral contraceptive users. They also linked these changes to a recent report of an association between oral contraceptive use and cardiovascular disease.[397]

These two studies from Wynn's group contain considerable information, much of which remains relevant to current concerns over oral contraceptive safety. For example, it was shown that most of the increase in triglycerides was in larger (S_f 100 to 400) VLDL. The importance of this observation, not to be recognised for many years, lay in the fact that the rise in triglyceride and VLDL levels differed in quality from the rises seen in those at risk of coronary heart disease. A shift was also demonstrated within the LDL spectrum towards denser particles. Almost 30 years later a major interest would develop in small dense LDL as a possible factor in the development of vascular disease, and the shift towards denser LDL particles in oral contraceptive users would be rediscovered.[398] These studies did not distinguish between the effects of different oral contraceptive formulations. At the time, these were rather similar, comprising an alkylated estrogen and an estrane progestogen. The dose of estrogen varied however (between 0.15 and 0.05mg), and in a subsequent report from Wynn's group it was noted that the increase in triglycerides was estrogen dose-dependent.[399]

1970s: Population-Based Studies in the US

This effect of estrogen dose was also apparent in studies published during the latter part of the 1970s.[400,401] These were based on the US Lipid Research Clinics (LRC) programme of investigation and as such may be especially reliable on account of the large numbers of women studied and the high standards of technical analysis and quality control. In these studies, lipid and lipoprotein levels from 2606 women attending 10 LRC centres were compared in 424 oral contraceptive users and 1575 controls. Findings from earlier studies, including higher plasma levels of total cholesterol, triglyceride and VLDL in users of higher estrogen dose formulations, were broadly confirmed. It is noteworthy that levels of LDL cholesterol were raised, but only in younger (15 to 34 years) women, an observation previously made in the reports from Wynn's group. Levels of VLDL cholesterol were raised in all ages. A further study emphasising the dose-dependency of the estrogen effect on triglycerides was that of Wahl et al.[402] published in 1983. These authors also found that oral contraceptives increased the triglyceride content of all lipoproteins, not just VLDL. Further analysis of this dataset[403] indicated a dissociation between variation in HDL and triglyceride levels in oral contraceptive users, in contrast to the normally close inverse relationship.

The LRC Studies were important in being the first to undertake measurement of HDL cholesterol in large numbers of oral contraceptive users. Surprisingly, no consistent effect was seen on HDL cholesterol levels. Moreover, there was no clear relationship between estrogen dose and levels of LDL or HDL. Comparisons regarding these lipoproteins may have been confounded by variation in progestogen dose, however, despite there being insufficient numbers of women using any given progestogen at a given dose to distinguish the effects of progestogens. In other studies, undertaken both concurrently and subsequently during the 1980s, more effective discrimination of the effects of the progestogen was possible, enabling the relative contributions of estrogen dose and, in particular, progestogen dose and type to changes in lipid and lipoprotein metabolism in oral contraceptive users. These studies were accompanied by a major shift of emphasis from the increases in levels of triglycerides and LDL to the fall in those of HDL, reflecting the increased recognition of the antiatherogenic role of this lipoprotein.[404] These investigations were facilitated by the development and validation of relatively inexpensive precipitation assays, al-

lowing the determination of HDL in large numbers of samples.

The Contraceptive Drug Study[405] involved members of the Kaiser Foundation Health Plan living in Walnut Creek, California, USA and was the first study to highlight relationships between progestogen content and HDL cholesterol level as being of potential importance in vascular disease risk in oral contraceptive users. Levels of HDL cholesterol in 592 women, using between them over 12 different oral contraceptive formulations, were compared with those in non-users. After standardisation of the data for potential confounding variables, it was found that some oral contraceptive formulations increased HDL cholesterol levels whereas others decreased them. This observation therefore helped account for earlier uncertainties regarding the effects of oral contraceptives on HDL levels. Levels of HDL cholesterol were positively associated with estrogen dose but negatively associated with the dose of progestogen.

1980s: Low Dose Formulations and the Effects of Different Progestogens

Throughout the 1980s and after, the great majority of studies have concerned formulations containing less than 0.05mg ethinylestradiol. Attention has therefore focused on variation in lipid and lipoprotein levels according to oral contraceptive progestogen content, both with regard to dose and type of progestogen. The large body of data on oral contraceptives and plasma lipoproteins generated by the numerous clinical trials and cross-sectional comparisons undertaken during this period have been reviewed in detail elsewhere[406-410] and only the broad pattern of changes will be described here.

The older low estrogen dose combined oral contraceptives containing levonorgestrel increased fasting triglyceride and LDL levels and reduced those of HDL. Reducing the levonorgestrel dose has resulted in lesser changes in LDL and HDL to the extent that triphasic levonorgestrel formulations have relatively little effect on either lipoprotein.[411] Similarly, high-dose norethisterone formulations can increase LDL levels and reduce those of HDL[412] but these changes can be minimised by dose-reduction. In formulations containing the lowest dose of norethisterone (0.5mg) the ability of the estrogen component to increase HDL levels may predominate.[411,413] Such an elevation in HDL levels is also seen in formulations containing the more recently introduced progestogens, desogestrel, gestodene and norgestimate. LDL levels may be slightly reduced with these formulations (especially in older women[414]) but, as would be expected, the estrogenic increase in triglyceride levels persists. A variety of formulations of similar low estrogen content (0.03 to 0.04mg ethinylestradiol) but differing in progestogen dose and type were evaluated in a single study.[411] The estrogen-induced rise in HDL was apparent with low-dose norethisterone and desogestrel, but this was opposed with increasing doses of levonorgestrel and norethisterone. With high and medium dose levonorgestrel and high dose norethisterone, HDL cholesterol levels were lower than those seen in controls. Desogestrel formulations containing ethinylestradiol 0.02mg have less effect on plasma lipoproteins than do those containing estrogen 0.03mg although in some studies this difference is rather less than would be expected.[415] Gestodene formulations have essentially the same impact on plasma lipoproteins as do those containing desogestrel, with perhaps rather less of an increase in HDL.[416] There are surprisingly few comparative studies on norgestimate formulations but the lipoprotein profile appears to resemble that seen with gestodene rather than levonorgestrel.[417]

Overall, these studies show that the effect of different oral contraceptives on plasma lipoproteins clearly reflects the balance of estrogen and progestogen, both in terms of steroid dose and the type ('androgenicity/anti-estrogenicity') of progestogen. In general, changes are fully expressed within 3 months of oral contraceptive use, do not progress with continued exposure and are fully reversed when therapy ceases.[406] Progestogen-only oral contraceptives have generally been found to have little or no effect on plasma lipoprotein levels.

4.3.3 The Clinical Significance of Oral Contraceptive-Induced Changes in Lipid and Lipoprotein Metabolism

Triglycerides

Fasting triglyceride levels predict arterial disease, especially in women.[418] Interpretation of studies in which fasting triglyceride levels are changed by diet or drugs are often confounded by changes in other lipoproteins, in particular an increase in HDL. Nevertheless in both the Helsinki Heart Study[419] and the more recent Bezafibrate Coronary Atherosclerosis Intervention Trial[420] it was possible to show a clinical benefit that could be related to the fall in triglyceride levels. It would therefore seem prudent to avoid elevations in triglyceride levels. Nevertheless, there are several arguments against the relevance of this case to oral contraceptive-induced increases in triglyceride levels. First, elevated fasting triglyceride levels increase the risk of arterial disease only if HDL levels are low; but with the majority of formulations in current use, HDL levels are unchanged or raised. Secondly, combined oral contraceptives increase fasting plasma triglyceride levels by increasing VLDL secretion, not by impairing catabolism.[421] It is the latter defect which is thought to contribute to arterial disease risk. In support of this argument, several drugs have been identified which elevate triglyceride levels but decrease rather than increase the risk of arterial disease. These include cholestyramine, ethanol and conjugated equine estrogens, and in each case the elevation in triglycerides they induce is due to an increasing in synthesis rather than a decrease in catabolism. Thirdly, there is increasing evidence that postprandial lipaemia may be of greater clinical importance than the fasting triglyceride level and combined oral contraceptives improve rather than impair the postprandial clearance of chylomicron remnants.[422]

Triglycerides and Haemostasis

Two concerns need to be acknowledged in this context. First, elevated plasma triglyceride levels are associated with abnormalities in the haemostatic system. These include an increase in FVII activation[423] and an increase in the activity of the antifibrinolytic factor PAI-1.[424] However, increased FVII activation appears to relate primarily to the presence of increased postprandial triglyceride-rich lipoprotein remnants, which are not increased in oral contraceptive users. PAI-1 levels are not increased by oral contraceptives, rather they are decreased. Combined oral contraceptives also carry the risk of idiosyncratic hypertriglyceridaemic responses due to their estrogen component.[425] This is a potentially catastrophic event, often presenting as severe pancreatitis. At present there is no evidence that oral contraceptive formulations which induce relatively high triglyceride levels increase the incidence of this very rare adverse effect beyond other formulations.

Low Density Lipoproteins

An increased LDL level is rather weakly associated with increased cardiovascular disease in women, in contrast to the stronger associations seen with increased triglycerides or decreased HDL.[426] The small (<5%) effects seen with currently used low estrogen dose formulations may be only of minor clinical relevance. With regard to the trend towards smaller, denser LDL seen in oral contraceptive users, similar changes are seen with unopposed estrogen therapy in postmenopausal women. The clinical significance of this change will require further exploration.

High Density Lipoproteins

Not only are high HDL levels associated with reduced risk of arterial disease in epidemiological studies, but interventions which increase HDL levels are also found to be protective. Such interventions have included drugs, lipoprotein infusions or genetic manipulations. Evidence for HDL as a major negative risk factor for arterial disease continues to accumulate, the latest findings being that pharmacologically-induced increases in HDL in men with low levels of HDL cholesterol are effective in the secondary prevention of coronary heart disease,[427] and that gene transfer of apoAI in animal models of atherosclerosis is effective in inducing regression of existing atherosclerotic lesions.[428] As HDL is a major protective risk factor for this disease in women, the 10 to 15% increase

induced by low estrogen dose oral contraceptives containing desogestrel, gestodene or norgestimate, or norethisterone 0.5mg would be expected to protect women against MI and other manifestations of arterial disease.

4.3.4 Conclusions

Depending on the doses and types of the component steroids, the oral contraceptives in current use induce a variety of changes in serum lipid and lipoprotein risk markers for cardiovascular disease. In general, there are variable increases in triglyceride levels, with no change in total cholesterol levels. Effects on LDL cholesterol are minor, but a broad range of effects on HDL cholesterol may be encountered. Older formulations (e.g. ethinylestradiol 0.03mg with levonorgestrel 0.15mg) tend to reduce HDL cholesterol levels, whereas in users of more recently introduced formulations (e.g. ethinylestradiol 0.03mg with desogestrel 0.15mg) these levels are increased. Recent research into interactions between lipoproteins and the vessel wall support the development of formulations which increase HDL levels although consistent epidemiological validation of this approach is still awaited.

4.4 Carbohydrate Metabolism

The clinical importance of carbohydrate metabolism has been viewed historically with regard to risk of diabetes mellitus. However, since the late 1960s there has been an increasing awareness that subclinical elevations in glucose levels and also in insulin levels may increase risk of arterial disease. This has culminated in the nomination of insulin resistance and its accompanying syndrome of metabolic disturbances (the metabolic or insulin resistance syndrome) as a primary factor in the development of arterial disease.[429]

The metabolism of carbohydrates to carbon dioxide and water is central to the body's energy economy, and the involvement of insulin in promotion of this process – or diversion of glucose into storage – is central to its control. The classic condition in which the normal relationship between glucose and insulin is disrupted is diabetes mellitus, insufficient insulin action – due to insulin deficiency or insulin resistance – being the common factor in all the forms that the disease takes. Diabetes mellitus, particularly type 2 (non-insulin dependent diabetes mellitus; NIDDM), carries with it a substantially increased risk of vascular disease. Importantly, the condition carries a 2- to 3-fold increase in risk of coronary artery disease. This increase is especially marked in women, who, after they develop diabetes mellitus, appear to entirely lose the protection from coronary heart disease that they have hitherto enjoyed relative to men.[430]

Changes in glucose tolerance diagnostic of diabetes mellitus have been reported in oral contraceptive users, particularly in those taking high estrogen dose combinations,[431] and impaired glucose tolerance and insulin resistance have been repeatedly described in oral contraceptive users.[382] Despite these changes the development of overt, clinical diabetes mellitus has not been linked with oral contraceptive use. In animal models, estrogens – including those used in combined oral contraceptives – protect against the development of diabetes mellitus by exerting a positive trophic effect on the pancreatic islet β cells.[432] Moreover, a recent analysis of data gathered as part of a large cross-sectional study noted that the age-related decline in glucose tolerance and first-phase insulin release seen in women (aged 18 to 44 years) was absent in oral contraceptive users.[433] Possibly oral contraceptive estrogens sustain the pancreas in the face of the stresses of impaired glucose tolerance and insulin resistance that these same steroids impose. These observations suggest that diabetes mellitus is unlikely to be a major issue in possible links between oral contraceptives and vascular disease. However, the subclinical disturbances in carbohydrate metabolism induced by oral contraceptives need to be considered.

Blood glucose and insulin levels are determined within a closed mutual feedback loop: a rise in glucose level stimulates the pancreatic β cell to release more insulin, insulin levels rise, glucose elimination increases, the β cells release less insulin and glucose and insulin levels return to basal.[434] A

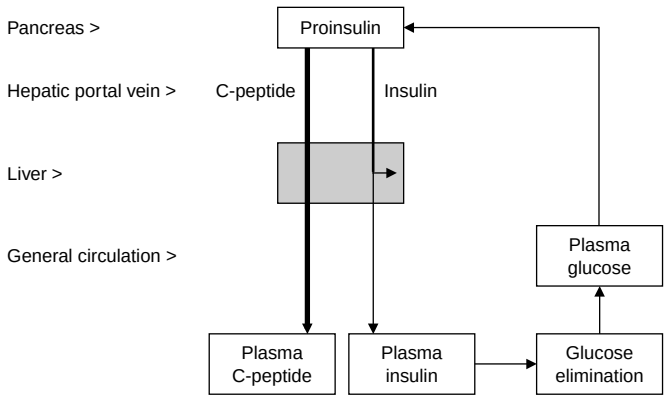

Fig. 17. The insulin/glucose feed-back loop: interrelationships between insulin, C-peptide and glucose.

number of factors dictate the 'set' of this feedback loop: the number of insulin-producing cells in the pancreas, the sensitivity of insulin release to glucose, the amount of newly-secreted insulin taken up by the liver in passing from the hepatic portal vein into the general circulation (about 50%), the plasma half-life of insulin, the ability of glucose to promote its own elimination by mass action, and the sensitivities of glucose disposal and glucose production to insulin. These factors are illustrated in figure 17, which also illustrates the place of C-peptide. C-peptide and insulin are the products of cleavage of the proinsulin molecule within the β cell. Both are released simultaneously and in equimolar quantities into the hepatic portal vein but, unlike insulin, C-peptide is not taken up by the liver.[435] Peripheral C-peptide levels therefore provide a more direct index of pancreatic insulin secretion than do insulin levels, and simultaneous evaluation of plasma C-peptide and insulin dynamics can provide information about hepatic insulin handling.

The main factor in subclinical variation in glucose and insulin levels appears to be variation in insulin sensitivity.[436] Both the sensitivities of stimulation of glucose disposal in peripheral tissues and of suppression of hepatic glucose production of insulin, appear to important in this respect. Basal glucose levels are maintained within rather tight limits, primarily by hepatic glucose production, which is itself largely determined by the insulin : glucagon molar ratio in the hepatic portal vein. If there is resistance to suppression of hepatic glucose production by insulin – as in obesity for example – there is a compensatory rise in the basal insulin output in order to maintain basal glucose levels within normal limits. Basal glucose levels do not rise beyond diabetic thresholds unless there is a substantial deficit (>85%) in functioning pancreatic β cells.[434]

There is appreciably greater variation in glucose levels after a glucose load than in the basal state. Disposition of glucose after loading is largely directed to peripheral tissues so variation in the sensitivity to insulin of glucose uptake by peripheral tissues will be accompanied by variation in post-load glucose and insulin levels. Where there is appreciable resistance to this action of insulin, the deterioration in glucose tolerance (i.e. the rise in glucose levels after loading) may exceed diagnostic levels for impaired glucose tolerance, although again development of diabetes mellitus requires there to be a substantial deficit in functioning β cells. As a rule, normoglycaemia appears to be maintained at the expense of compensatory hyperinsulinaemia. Thus, in states of insulin resistance such as obesity there may be a considerably greater degree of hyperinsulinaemia than hyperglycaemia

following a glucose load. Such associations underlie the primary role of variation in insulin resistance in subclinical variation in insulin levels.

Three factors must be considered with regard to subclinical variation in carbohydrate metabolism: hyperglycaemia, hyperinsulinaemia and insulin resistance. Most epidemiological studies of these factors have examined risks of MI. In the following sections, evidence implicating each factor in the development of MI and vascular dysfunction is summarised, the effects of oral contraceptives on each factor are assessed, and the clinical significance of these changes is considered.

4.4.1 Hyperglycaemia

Hyperglycaemia and Vascular Disease

Hyperglycaemia here refers to elevations in fasting or post-load glucose levels within ranges below those diagnostic for diabetes mellitus (according to WHO criteria: fasting glucose levels <7.0 mmol/L, and 2-hour post-load glucose levels of <11.1 mmol/L). In view of the close metabolic relationships that exist between plasma glucose, insulin levels and insulin resistance, any consideration of hyperglycaemia in isolation will be somewhat artificial. Moreover, current interest in glucose and insulin as risk factors for vascular disease centres very much on insulin and insulin resistance. Nevertheless, the potential importance of subclinical hyperglycaemia in the development of cardiovascular disease has been emphasised.[437]

An early attempt to link subclinical hyperglycaemia and the development of vascular disease was made in 1979 with a meta-analysis of epidemiological evidence.[438] This indicated that there was little risk of coronary heart disease associated with subclinical increases in fasting or post-load glucose levels. However, subsequent studies and re-analyses of some of the studies included in the 1979 meta-analysis (but with longer follow-up times and greater numbers of events) suggest that such an association does exist.[439,440]

Two levels of subclinical hyperglycaemia may be distinguished: impaired glucose tolerance, which has its own diagnostic criteria and may be considered as a distinct clinical condition; and dysglycaemia involving lesser degrees of hyperglycaemia.[437] The Honolulu Heart Study provides some of the strongest evidence for a link between impaired glucose tolerance and vascular disease,[441,442] but has also highlighted possible risks associated with milder forms of hyperglycaemia. Among the 8006 men included in this study, the 80% of individuals who had a glucose level >6.4mmol/L one hour after a 50g oral glucose load had an 82% increase in attributable risk of coronary heart disease compared with those with lesser increases in post-challenge glucose levels.[443] Other studies have also found increased risk of vascular disease in those with mild degrees of hyperglycaemia.[444-446]

Candidate mechanisms by which hyperglycaemia could promote vascular disease include protein glycation,[447] glucose toxicity[448] and free radical generation.[449] Whether these mechanisms are important at sub-diabetic degrees of hyperglycaemia is unclear. Glycosylated proteins, or advanced glycosylation end-products, may accumulate with even the minor degrees of hyperglycaemia seen with aging.[450] Such proteins can disrupt normal endothelial function by increasing endothelial permeability and potentiating the cytokine-mediated transition from anti- to pro-coagulatory endothelial function.[451]

Hyperglycaemia in Oral Contraceptive Users

General Effects

Deterioration in glucose tolerance in women taking combined oral contraceptives was first reported by Waine et al.[452] in 1963. Since then there have been reports of significantly elevated post-load plasma glucose levels in users of virtually all types of combined oral contraceptive, from the highest to the lowest estrogen doses and with almost all types of progestogen.[453-458] An interesting exception to this rule appeared to be a combination containing the pregnane, megestrol[459,460] which may actually correct the impairment of glucose tolerance induced by ethinylestradiol.[461] With some of the earlier, high estrogen dose combinations (>0.05mg mestranol or ethinylestradiol) a diabetic level of glucose intolerance could de-

velop,[431] but such marked hyperglycaemia appears to have been eliminated by subsequent reductions in estrogen dose.[458]

Changes in Mean Values and Prevalence of Outliers

With regard to the subclinical changes in glucose tolerance seen with medium and low estrogen dose combinations, it has been difficult to obtain an accurate picture of their overall magnitude since many studies have been relatively small and designed primarily to develop the safety profile of a particular combination. In some cases, results have simply been summarised in phrases such as, 'all changes remained within the normal range', i.e. none of the women became overtly diabetic. Alternatively, and most commonly, results have been expressed as mean values at each time point during the glucose tolerance test, or in terms of the mean of a summary measure of glucose levels during the glucose tolerance test [e.g. area under the curve (AUC)]. In most studies, differences in mean values provide the only way of assessing the magnitude of oral contraceptive-induced changes. However, particularly in small longitudinal studies, changes in these mean values will only relate to the few women participating. In the global population of oral contraceptive users there will be considerable variation in individual response and there may be significant increases, not only in the mean value, but also in the prevalence of individuals with degrees of deterioration in glucose tolerance at the upper extreme of the frequency distribution. As described above, some epidemiological studies suggest these individuals would be most at risk. An analysis of data from our large cross-sectional study revealed that there was indeed a substantial increase in the prevalence of such individuals.[462] Using the 95th percentile limit for the oral glucose tolerance test (OGTT) 120 minute plasma glucose levels in 639 healthy premenopausal women not taking oral contraceptives, it was found that the proportion of outlying values in 229 women taking a combination of ethinylestradiol 0.03mg and levonorgestrel 0.15mg was increased 2.5-fold. As the glucose level limit used in the cross sectional analysis was relatively low (since the non-users were required to be healthy, non-obese and less than 45 years of age) the clinical significance of this finding remains unclear. Nevertheless, the 2.5-fold increase in prevalence agrees well with the United States National Health and Nutrition Examination Survey (NHANES: 1976 to 1980) figure for the prevalence of impaired glucose tolerance in oral contraceptive users: 15.4% compared with 6.3% in non-users.[463] The analysis also illustrates the point that significant increases in mean glucose values do not only relate to a shift in the modal value of the frequency distribution, but also involve a shift in its upper tail.

Effects of Duration of Use

Another factor of potential importance in the net impact of oral contraceptives on glucose tolerance is the effect of increasing duration of use. In a large longitudinal study of the effects of a combination containing ethinylestradiol 0.03mg and levonorgestrel 0.15mg, glucose tolerance declined progressively up to 3 years.[464] However, in a subsequent large cross-sectional study, which used the same measurement methodology, changes in glucose tolerance in 214 users of this combination showed no further progression beyond 3 years: the mean OGTT incremental glucose AUC was virtually the same among women using this combination for ≤3 years, 3 to 6 years, 6 to 9 years and 9 to 12 years.[433,465] Either the maximum effect of the combination is present at 3 years or there is self-selection after 3 years whereby women whose glucose tolerance might deteriorate still further choose, for whatever reason, to discontinue this combination. Whichever explanation is true, it is unlikely that such effects will have major long term clinical implication beyond the effects of the deterioration in glucose tolerance seen at 3 years.

Changes in Fasting Levels

The above discussion of oral contraceptive effects on glucose tolerance has referred to deterioration in post-load glucose tolerance. It is important to note that there has never been any consistent reporting of increased fasting glucose levels in oral contraceptive users, rather the opposite appears to be true in that fasting glucose levels tend to be re-

duced. This almost certainly results from the specific antagonism that estrogens exert both to the action of and secretion of glucagon. This effect has been reported by several groups,[466-469] its net result being a marked increase in the basal hepatic portal insulin : glucagon molar ratio and a consequent reduction in the basal activity of the critical gluconeogenic enzyme, phosphoenolpyruvate carboxykinase which results in a reduction in basal glucose levels.[470]

Potential Mechanisms

The mechanism of oral contraceptive-induced deterioration in glucose tolerance remains unclear, although careful review of the literature reveals estrogen-induced increases in corticosteroid activity to be the most likely candidate.[382] The degree of hyperglycaemia induced by combined oral contraceptives is unusual, however. In a comparison of low estrogen dose oral contraceptive users with non-users who were either moderately overweight or of normal bodyweight, deterioration in glucose tolerance was significantly greater in the oral contraceptive users than in the moderately overweight women, whereas the latter had the greatest elevation in insulin (IF Godsland and V Wynn, unpublished data). Thus, in obesity, there may be sufficient compensatory hyperinsulinaemia to re-establish normoglycaemia, but this is not the case in oral contraceptive users. Corticosteroids appear to have a biphasic effect on pancreatic insulin secretion, with short term suppression but long term potentiation. In this respect, the study of Watanabe et al.,[471] in which 130 users of two different levonorgestrel-containing combinations were compared, is of interest. The authors concluded that in oral contraceptive users, insulin delivery was inappropriately low for their degree of post-load hyperglycaemia.

Clinical Significance

The clinical significance of oral contraceptive-induced changes in glucose levels is difficult to evaluate. Theoretically, any increase in glucose levels could increase the accumulation of advanced glycosylation end products, but whether this is the case in oral contraceptive users is unknown. There is evidence that glycosylated haemoglobin is unaffected. It is possible that, despite deterioration in glucose tolerance, the accompanying reduction in basal glucose levels minimises protein glycosylation.

4.4.2 Hyperinsulinaemia

Hyperinsulinaemia and Vascular Disease

Epidemiological evidence links hyperinsulinaemia with the development of vascular disease.[472-475] In two studies, glucose ceased to be a significant predictor of vascular disease when fasting and post-load insulin levels were included in the multivariate analysis.[472,473,476] It may be unrealistic to single out independent predictors in this way when dealing with highly correlated variables such as glucose and insulin. There is nevertheless experimental evidence for a direct involvement of insulin in the development of vascular disease (see later this section). It is possible that, in those studies which identified hyperglycaemia as a significant predictor for the development of coronary heart disease, it was primarily acting as a surrogate measure for hyperinsulinaemia, the two appearing together as an expression of insulin resistance.

In addition to being a predictor of the development of arterial disease, hyperinsulinaemia is a well established feature of existing cardiovascular disease, including primary hypertension, angiographically-established coronary artery disease,[477] chronic heart failure[478] and cardiological syndrome X (history of angina-like chest pain, an ischaemic exercise electrocardiogram and angiographically-normal coronary arteries).[479,480]

Experimental work implicating insulin in some of the early events in the development of vascular pathology dates back to the late 1960s with the work of Stout et al.[481,482] on stimulation of smooth muscle cell proliferation and arterial lipid deposition by insulin. It is also noteworthy that earlier studies of animal models of coronary disease demonstrated that insulin could oppose the protective effects of estrogen.[483] More recently, insulin has been shown to stimulate the synthesis of the antifibrinolytic factor, PAI-1,[484] and, although more controversial, there is evidence that insulin can stimulate triglyceride release from the liver[485] and increase BP by increasing catecholamine lev-

els.[486] There is also the possibility that insulin can stimulate the activity of hepatic lipase, thus reducing HDL_2 levels,[487,488] and there is evidence for a link between hepatic insulin handling and HDL_2 levels.[489] Moreover, interrelationships between insulin and endothelial function have been reported,[490] and insulin can stimulate release of the potent vasoconstrictor, endothelin-1.[491]

Such epidemiological and experimental observations make insulin a strong candidate for risk factor status, but recently this possibility has come under strong criticism.[492,493] The principal concern is that the epidemiological studies which show insulin to be a significant predictor of arterial disease are in a minority.[494] In defence of the importance of insulin as a risk factor, it must be emphasised that the positive studies are generally the most powerful in terms of study design. The negative studies may all be criticised for low numbers of cases, selected study groups or confounding by other factors.[495] There may, nevertheless, be threshold effects whereby the degree of hyperinsulinaemia prevalent in the group under consideration must exceed a certain level for increased risk to be apparent.[473]

Almost all epidemiological information regarding insulin and vascular disease relates to men, although a recently published prospective analysis suggests that insulin may also be a significant predictor in women.[475] A further strand of evidence relates to the long term health consequences of polycystic ovarian syndrome, which appears to have its origins in a state of hyperinsulinaemia and insulin resistance. One retrospective study suggests an increased risk of vascular disease in these women.[496] Connections between hyperinsulinaemia and the development of vascular disease in young women may, nevertheless, be confounded by the association between bodyweight loss and cigarette smoking.

Hyperinsulinaemia in Oral Contraceptive Users

General Effects and Differences between Formulations

Increased insulin levels accompanying oral contraceptive-induced post-load hyperglycaemia were first reported by Spellacy et al.[497] in 1966, and have been repeatedly confirmed. Comparative studies of hyperinsulinaemia in users of different oral contraceptive formulations are relatively scarce, and studies with the large numbers necessary to take into account the high variability in insulin levels are even rarer. The largest study of insulin levels in oral contraceptive users was reported by Wynn et al.[498] in 1979 and compared 577 women wishing to start oral contraceptive therapy with 1628 women currently taking various combined oral contraceptives. The oral contraceptive users were divided into 6 groups according to the estrogen dose and progestogen content of the combined formulations. OGTT glucose and insulin levels were raised in all groups except one (comprising women taking an ethinylestradiol/megestrol combination). The group of women taking high estrogen dose combinations (>0.05mg), was distinguished by the most severe deterioration in glucose tolerance, and by blunting of the initial insulin response and a significant reduction in the ratio of OGTT glucose to insulin response. These differences suggest that, although there was an overall increase in insulin response, there was a diminished sensitivity of pancreatic insulin release to glucose, and recalls the possible acute suppression of pancreatic insulin release mentioned in the previous section. The converse appeared to be the case with the levonorgestrel-containing combinations which were associated with the greatest increase in mean post-load insulin levels (fig. 18).

These effects were fully confirmed by Wynn's group in longitudinal studies of 421 users of combined oral contraceptives evaluated before and after at least 3 months on oral contraceptive therapy,[499] and the relative hyperinsulinaemia seen with levonorgestrel combinations was again seen in a later comparative study.[411] In this latter study, 346 non-users were compared with 675 users of combined oral contraceptives containing between 0.03 and 0.04mg ethinylestradiol. Seven combinations were compared, the progestogens being levonorgestrel or norethisterone, each at 3 different doses, or desogestrel. All formulations caused similar degrees of deterioration in glucose tolerance, but when

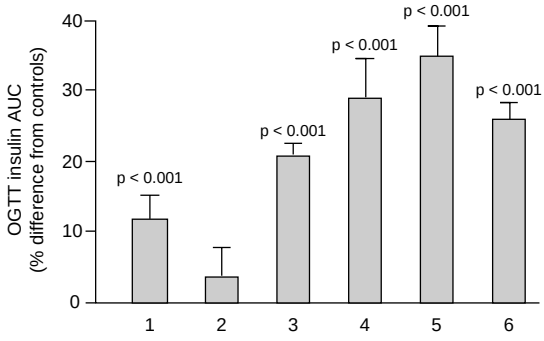

Fig. 18. Oral glucose tolerance test (OGTT) glucose and insulin responses in 1628 oral contraceptive users compared with 577 non-users (controls).
Formulations studied were: 1) mestranol 0.075-0.15mg with 1.0-5.0mg of estrane progestogen; 2) ethinylestradiol 0.05mg with megestrol 4.0mg; 3) ethinylestradiol or mestranol 0.05mg with 1.0-4.0mg of estrane progestogen; 4) ethinylestradiol 0.05mg with levonorgestrel 0.25mg; 5) ethinylestradiol 0.03mg with levonorgestrel 0.25mg; 6) ethinylestradiol 0.03mg with levonorgestrel 0.15mg (reproduced from Wynn et al.,[498] with permission). **AUC** = area under the curve, a mean summary measure.

OGTT insulin and C-peptide responses were considered, marked differences were apparent. Among the levonorgestrel-containing formulations OGTT insulin response increased with increasing progestogen dose, and a similar relationship was seen with OGTT C-peptide response among the norethisterone-containing combinations. The desogestrel combination was distinguished by a relatively high mean insulin response but with little effect on C-peptide. These relative differences between levonorgestrel, norethisterone and desogestrel combinations were confirmed in a parallel study in which intravenous glucose tolerance test (IVGTT) glucose, insulin and C-peptide responses in 95 non-users were compared with 266 combined oral contraceptive users.[500] In subsequent studies deterioration in glucose tolerance and hyperinsulinaemia were also seen with formulations comprising ethinylestradiol 0.03mg with gestodene 0.075mg, and ethinylestradiol 0.02mg with desogestrel 0.15mg.[415,416]

Changes in Mean Values and Prevalence of Outliers

The large group sizes and cross-sectional design of the studies described above has enabled one further concern to be addressed, namely the prevalence of abnormally elevated insulin responses to glucose. Using the OGTT incremental insulin AUC as a measure of insulin response it was found that the prevalence of insulin responses above the 95th percentile cut-off (defined in the non-user control group) was markedly increased among the low-estrogen dose oral contraceptive users.[405] The extent of this increase varied markedly with progestogen content, however: the prevalence of abnormally high insulin responses was increased 5- to 7-fold among the levonorgestrel combination users, and was levonorgestrel dose-dependent, but the prevalence was only increased 2-fold among users of a low dose norethisterone and a desogestrel-containing combination.[382] This latter combination was interesting since its use was accompanied by an appreciable increase in mean OGTT insulin response despite showing the least increase in the proportion of outlying values.[501] This dissociation between mean value and proportion of outlying values was not seen with any of the other combinations, and may be due to an effect on plasma insulin half-life.[382]

Clinical Significance

With regard to the clinical significance of these changes described, several mechanisms have been proposed by which hyperinsulinaemia could promote vascular dysfunction. This possibility is strengthened by the observation that it is not only shifts in the distribution of insulin responses 'within the normal range' that are being seen; there are, with some formulations, substantial increases in the prevalence of outlying values. Moreover, interrelationships with other risk factors may exist. In an analysis of correlations between OGTT insulin response and other potential vascular risk factors in combined oral contraceptive users and non-user controls, stronger associations were found between insulin response and BP and HDL cholesterol in oral contraceptives users than in non-user controls.[502] Only the association between insulin response and serum triglyceride level was diminished in oral contraceptive users.

The weight of evidence suggests that it would be prudent to avoid hyperinsulinaemia in oral contraceptive users if possible. Hyperglycaemia, and insulin resistance (see section 4.4.3), appear to be inevitable consequences of the use of the potent alkylated estrogen, ethinylestradiol, as is some degree of hyperinsulinaemia. However, the magnitude of the hyperinsulinaemia does appear to be modifiable by alteration in progestogen type and dose.

4.4.3 Insulin Resistance

Insulin Resistance and Vascular Disease

Insulin resistance is unusual among potential vascular risk factors in that it may operate primarily through secondary effects. It might be argued that resistance to the vasodilatory effects of insulin and limitations on the fuel supply to the myocardium consequent on impaired glucose uptake constitute direct adverse effects on the vasculature. However, it is recognition of the manifold adverse influences of insulin resistance on other cardiovascular risk factors that has been responsible for the prominence given to insulin resistance as a risk factor for arterial disease.[503,504]

Resistance to peripheral glucose elimination and suppression of hepatic glucose production has been mentioned in section 4.4.2 in relation to compensatory hyperinsulinaemia. As described, this compensatory hyperinsulinaemia may have direct adverse effects on the vessel wall, and may increase catecholamine activity and BP, hepatic triglyceride release and synthesis of PAI-1. There may also be resistance to suppression of adipose tissue lipolysis by insulin. This can lead to increased supply of NEFA to the liver, increased synthesis and release of triglyceride-enriched VLDL, an increased proportion of small, dense LDL and decreased levels of HDL. Each of the lipoprotein disturbances engendered in this insulin resistance-related dyslipidaemia has been linked with development of vascular disease. Another feature of insulin resistance is resistance to the vasodilatory action of insulin, a characteristic which may, in combination with insulin-stimulated catecholamine activity, lead to the association that has been identified between insulin resistance, hyperinsulinaemia and hypertension.[503,504]

The list of adverse disturbances in vascular risk factors that have been linked with insulin resistance grows each year. In addition to those mentioned above, decreased postprandial triglyceride elimination, increased uric acid levels and increased centrally distributed body fat may also be included. The correlations that have been observed between insulin resistance and these various disturbances, and the mechanistic connections that may underlie them, has led to adoption of the concept of a distinct 'metabolic' or 'insulin resistance' syndrome.[369] It must be kept in mind that, as a concept, the insulin resistance syndrome is at a relatively early stage of development, and already there is evidence that the associations on which the concept is based are not as straightforward as has been proposed. Because of the difficulties in carrying out such studies, insulin resistance has yet to be evaluated as a predictor of cardiovascular disease and, with just two exceptions,[372,505] this also applies to quantitative indices of the severity of the accompanying syndrome of metabolic disturbances. Of more concern is the recognition that insulin resistance is not invariably accompanied by all proposed features of the insulin resistance syndrome. People from the Indian subcontinent are at increased risk of coronary heart disease and show many of the features of the insulin resistance syndrome, but do not have increased BP. Patients with cardiological syndrome X are insulin resistant, but do not appear to experience an increased incidence of coronary disease and do not show all features of the insulin resistance syndrome.[480] Postmenopausal women exhibit many of the features of the insulin resistance syndrome, but estrogen deficiency appears to be at the root of the disturbances and other changes not associated with insulin resistance (e.g. increased LDL levels, decreased pancreatic insulin secretion) are present.[506] It may be more realistic to think in terms of *syndromes* of risk factor disturbance rather than a single syndrome. The risk factor disturbances associated with oral contraceptive

use might be considered as a separate class of metabolic syndrome.

Insulin Resistance and Oral Contraceptive Therapy

General Effects

There have been very few studies in which insulin resistance has been directly measured in oral contraceptive users. The first formal study, that of Srivastava et al.[378] in 1975, assessed insulin resistance in terms of the magnitude of the hypoglycaemic response to insulin. This study is problematic since the 6 oral contraceptive users were studied compared with a control group of 5 men and 5 women. Nevertheless, insulin sensitivity was reduced by the medium estrogen dose, estrane progestogen combinations that were studied. Singh and Nattrass[507] also evaluated insulin resistance in oral contraceptive users by assessing the hypoglycaemic action of insulin. In this study, the effects of successively higher insulin infusion rates were compared in 8 women taking levonorgestrel- or norethisterone-containing combined oral contraceptives, compared with 8 non-user controls. There was a small but significant reduction in the degree of insulin-induced hypoglycaemia in the oral contraceptive users.

Two studies have employed the euglycaemic, hyperinsulinaemic clamp technique to measure insulin resistance in oral contraceptive users. Skouby et al.[508] observed a reduction in glucose infusion rate from 1.56 to 1.30 mmol/m^2/min in healthy women and 1.51 to 1.10 mmol/m^2/min in women with previous gestational diabetes mellitus after 6 months of taking a low dose triphasic oral contraceptive combination containing ethinylestradiol and levonorgestrel. There was no change in OGTT glucose or insulin response during oral contraceptive therapy, although in the group of normal controls there were strong correlations between OGTT insulin response and steady-state glucose infusion rates at 2 and 6 months on oral contraceptive therapy.[509] Using the euglycaemic clamp with stepped insulin infusion rates of 10 and 40 mU/m^2/min, Kasdorf and Kalkhoff[510] studied 7 healthy women before and after 3 and 6 months of taking a low estrogen dose, levonorgestrel-containing combination. The glucose infusion rate was significantly reduced at 3 months during the high insulin dose phase, and the infusion rate divided by the steady-state insulin level was significantly reduced at 3 months during both insulin infusions, indicating that there was resistance to insulin-induced peripheral glucose elimination after 3 months of oral contraceptive use. These measures returned to baseline values after 6 months.

A problem with these studies has been low numbers involved (generally less than 10 per group). The advent of mathematical modelling analysis of IVGTT glucose and insulin levels has enabled measures of insulin sensitivity to be derived in substantially larger groups of oral contraceptive users. In the large cross-sectional comparative study mentioned above, there were 95 non-user controls and 296 oral contraceptive users.[500] The formulations compared were two different levonorgestrel-containing combinations, a desogestrel combination, a norethisterone combination, and formulations comprising norethisterone or etynodiol alone (considered together since the latter is metabolised to the former before becoming metabolically active). Despite variation in IVGTT insulin responses, all combinations caused a similar decline in glucose elimination rate and a similar degree of insulin resistance, whereas the estrane progestogen-only formulations had no effect. This was consistent with the possibility that the estrogen component was responsible for the insulin resistance (fig. 19). However, there did appear to be some variation according to progestogen content. A multivariate analysis was undertaken in which insulin sensitivity was predicted from estrogen dose (all combinations contained ethinylestradiol), net progestogenicity and androgenicity (estimated from published figures for progestogenic and androgenic activity), BMI, cigarette smoking, exercise habit and alcohol intake.[382] The strongest correlate of insulin sensitivity was oral contraceptive estrogenicity ($r = -0.27$, $p < 0.01$) followed by percentage of ideal bodyweight ($r = -0.15$, $p < 0.01$) and aerobic exercise ($r = 0.09$, $p = 0.08$). Neither

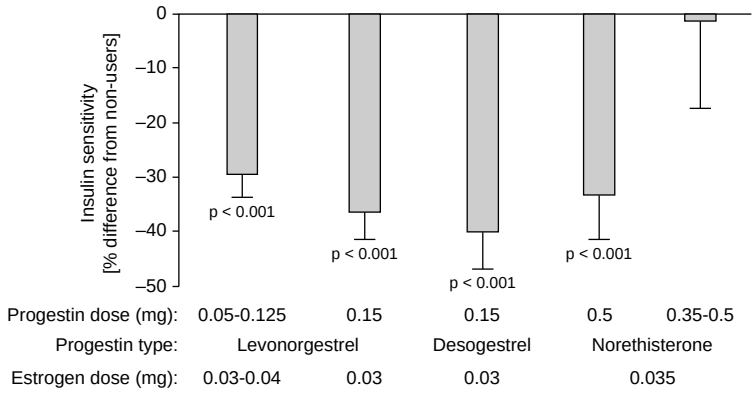

Fig. 19. Effects of different oral contraceptive formulations on insulin sensitivity measured by modelling analysis of intravenous glucose tolerance test insulin and glucose levels. 296 oral contraceptive users were compared with 95 non-users (controls) [reproduced from Godsland et al.,[500] with permission].

net progestogenicity or androgenicity influenced insulin sensitivity in this model.

Potential Mechanisms

As described above, oral contraceptive-induced insulin resistance may be explained by estrogen-induced increases in corticosteroid activity. A number of mechanisms have been proposed to account for insulin resistance in the context of increased risk of vascular disease. These include impaired transendothelial insulin transport, impaired action of insulin-sensitive enzymes of glucose metabolism, impaired intracellular signalling mechanisms, and metabolic competition from NEFA. So far, increased corticosteroid activity has not emerged as a prominent candidate. Therefore, there is still uncertainty over whether insulin resistance in oral contraceptive users has the same adverse consequences as the insulin resistance that has been linked with vascular disease. At least two of the features of the insulin resistance syndrome, decreased postprandial triglyceride elimination and increased PAI-1 activity, are not seen in oral contraceptive users, rather the opposite effects have been reported, and, with some formulations, HDL levels may be increased rather than decreased. Moreover, neither increased uric acid levels nor increased android body fat distribution have been found in oral contraceptive users. Analysis of correlations between insulin resistance and other metabolic risk factors in the cross-sectional study described above showed none of the associations characteristic of the insulin resistance syndrome. The weight of evidence therefore suggests that the insulin resistance seen in oral contraceptive is only important to the extent that it provokes the hyperinsulinaemic response to a glucose load. It is this hyperinsulinaemia that correlates with other risk factors in oral contraceptive users and which remains a potential risk factor for vascular disease, regardless of the mechanisms that underlie its appearance.

4.5 Blood Pressure (BP)

Increased BP is a classic risk factor for arterial disease, having been first fully identified as a predictor of MI and stroke, and risk of these diseases then having been diminished by specific pharmacological interventions designed to reduce BP. The pathogenetic effect of high BP has generally been ascribed to mechanical damage to the vessel wall. Thus, for example, Russell[511] ascribed the action of BP in the causation of stroke to mechanical distension of small resistance arteries that could give rise to aneurysm formation, plasma insudation into the vessel wall, with eventual occlusion or rupture. More recently, increased BP has been identified as a possible accompaniment of the metabolic distur-

bances associated with insulin resistance, raising the possibility that, in addition to mechanical damage, other pathogenetic processes may contribute to arterial disease in hypertension.

4.5.1 Elevated BP in Oral Contraceptive Users

Case studies conducted during the latter part of the 1960s showed that combined oral contraceptives could cause hypertension in some women, and could aggravate preexisting hypertension in others.[512-515] During the 1970s, larger prospective and cross-sectional comparisons confirmed these findings and suggested that, in addition to effects in susceptible individuals, there were mean increases of 5 to 7mm Hg in systolic BP (SBP) and 1 to 2mm Hg in DBP in oral contraceptive users in general.[20,193,516-518] These effects were seen in the majority but not in all studies.[519,520] Since 1980, the majority of studies have shown relatively few significant effects.[521-526] However, in some larger comparative studies significant increases have been reported.[527-530]

Although mean BP levels in users of currently-prescribed oral contraceptive formulations may be somewhat elevated, published reports of severe hypertension no longer appear in the literature. Furthermore, hypertension has not been a feature of the more recent clinical surveys.[531-535] These developments are probably attributable to a greatly increased awareness of the need for monitoring BP. A further contributing factor may be the reductions in steroid dose in the great majority of combinations currently prescribed.

Effects of Duration

In the first report from the RCGP study, a marked increase in the development of hypertension with increasing duration of use was apparent.[20] There have been no further reports of such a relationship, and in a recent analysis from a large cross-sectional study no significant relationship was found between BP and duration of oral contraceptive use.[433] Most studies suggest that elevated BPs are not apparent in ex-users,[513,518,536,537] although some residual increase after 8 months of use was apparent in one study.[529]

Joint Effects with Other Risk Factors

The joint effects of oral contraceptive use and other factors in the development of elevated BP have been evaluated in some studies. Petitti et al.[538] concluded that obesity and age over 35 years were the principal risk factors for hypertension in oral contraceptive users. Tsai et al.[539] found no difference in change in BP between women with a history of hypertension compared with women without such a history, when given a combination of ethinylestradiol 0.035mg and the relatively low progestogen dose of norethisterone 0.4mg.

Bias in Clinical Studies

It is conceivable that biases inherent in the designs of the studies described above in this section might contribute to the observed increase in BP in oral contraceptive users. For example, there might be a positive interaction between oral contraceptive use, cigarette smoking and the stress accompanying the clinic visit.[540,541] However, in the large cross-sectional study described above, which found elevated BP in users of levonorgestrel combinations, smokers were evenly distributed among the groups compared, and no effect of current smoking was apparent in multivariate analysis.[433] Conversely, a factor that might have diminished the magnitude of any observed increase in BP is the decline in such measurements that is independently associated with repeated clinic visits.[542,543] This might have contributed to the relative lack of effect reported in some of the large clinical trials, but is unlikely to have affected cross-sectional comparisons.

4.5.2 BP and Oral Contraceptive Steroid Content

Only a few studies have considered the effect of estrogen dose alone on BP. In the first report from the RCGP study the relative risk for the development of hypertension was 0.5 in users of formulations containing between 0.075 to 0.15mg compared with users of estrogen 0.05mg formulations.[20] This comparatively low figure probably related to increased awareness of BP problems after use of the higher dose formulations had almost disappeared. A further contributing factor is likely to have been the relatively lower progestogen doses

in the high estrogen dose oral contraceptives compared with the 0.05mg formulations. The WHO Multicentre Trial of the Vasopressor Effects of Combined Oral Contraceptives appears to be the only other study in which the effects of different doses of estrogen (ethinylestradiol 0.05 or 0.03mg), in the presence of the same progestin dose (levonorgestrel 0.25mg), were compared. No benefit of reduction in estrogen dose was apparent.[528]

With regard to progestogen dose, the Walnut Creek Contraceptive Drug Study found no significant effect in formulations containing predominantly estrane-type progestogens, although in this study 16 different formulations were included and both type and dose of both the estrogen and progestogen varied simultaneously.[544] Other studies, including the RCGP Study, have found evidence for increasing BP with increasing doses of the estrane progestogen, norethisterone, in formulations containing ethinylestradiol 0.05mg.[20,193,537,545] In the study of Weir et al.[537] this effect was not significant and was not seen with another estrane progestogen, lynestrenol. In these studies, doses of norethisterone ranged between 1.0 and 4.0mg. In a more recent comparative cross-sectional analysis,[530] use of formulations containing a lower range of doses (0.5 to 1.0mg) in combination with ethinylestradiol 0.035mg was not associated with significantly higher mean BP compared with non-users, although the lowest mean BP was still seen in users of the formulation containing the lowest norethisterone dose.

Several studies have examined BP in women taking formulations containing the gonane progestogen, levonorgestrel. In the study of Meade et al.,[545] mean BP in women taking ethinylestradiol 0.03mg with levonorgestrel 0.15 or 0.25mg were higher than those in users of combinations of ethinylestradiol 0.05mg with norethisterone 3 to 4mg. Khaw and Peart[527] compared women taking formulations containing ethinylestradiol 0.03mg and either levonorgestrel 0.15 or 0.25mg, and found higher mean BP at the higher progestogen dose. Nichols et al.[529] reported a comparison of the effects of 4 different formulations each containing ethinylestradiol 0.03mg. Progestogens were levonorgestrel (0.15mg), desogestrel (0.15mg), gestodene (0.075mg) and norethisterone (1mg). Group sizes ranged from 29 to 35 and BP were measured before, for 6 months during and 8 months after oral contraceptive use. Mean BP rose during oral contraceptive use in all groups, on average by 6.3mm Hg systolic and 6.4mm Hg diastolic. The greatest mean rise in SBP was seen in the levonorgestrel group (+8.4mm Hg) and in DBP in the desogestrel group (+10.0mm Hg). This study is unusual for the magnitude of the increases in DBP reported, none of which were significant. Mean BP fell after cessation of oral contraceptive use but did not return to baseline values. As in the study of Khaw and Peart,[527] a dose-dependent effect of levonorgestrel was also seen in the large comparative study previously mentioned in relation to lipid and carbohydrate metabolism, when users of a medium-dose, monophasic and low-dose, triphasic levonorgestrel-containing combination were compared.[530] Both mean SBP and DBP were higher at the higher levonorgestrel dose. Moreover, mean DBP with the medium estrogen dose, monophasic levonorgestrel combination was greater than that seen with a desogestrel combination of identical progestogen dose and estrogen content, use of which was not associated with a significant increase in BP. The adverse effect of levonorgestrel-containing formulations on BP was also apparent in a study of cardiovascular reactivity to stress, in which vascular resistance and BP increased during stress more in users of low-estrogen dose formulations containing levonorgestrel compared with those containing desogestrel.[546] This differential was particularly marked in cigarette smokers.

These findings generally support some role for the progestogen in oral contraceptive-induced elevations in BP, particularly in the case of the progestogen, levonorgestrel. Increased BP has not been found in previous studies examining the effect of orally administered progestogens given alone,[547,548] but progestogen-only contraception generally involves substantially lower doses than with even the lowest dose combination therapy. As described in

section 4.5.3, there is, nevertheless, experimental evidence for a role for the estrogen component.

4.5.3 Mechanisms

Supporting a causal relationship between oral contraceptive use and increased BP, oral contraceptive estrogens have been observed to induce adverse changes in factors that might affect BP. Estrogen-induced increases in blood volume and sodium retention,[549,550] aldosterone secretion and excretion,[551,552] renin substrate level and renin activity[552] have been described, and similar findings have been reported in oral contraceptive users.[513,515,536,553-555] However, women who become hypertensive on taking oral contraceptives have the same changes in these factors as women who remain normotensive[513] and these changes do not correlate with changes in BP.[523,556] Haemodynamic effects, including an increase in cardiac output, blood volume and stroke volume, have also been seen in oral contraceptive users.[554,557-559] These effects were associated with the estrogen rather than the progestogen component and were not necessarily associated with an increase in BP. Measurement of renin and prorenin levels and renin activity indicates that, in general, women taking oral contraceptives appear able to suppress renal release of active renin in response to estrogen-induced increases in renin substrate release.[560] It is then possible that susceptible individuals may lack the ability to suppress active renin levels in the presence of these increased renin substrate levels,[561] and it may be the lack of renin level measurements in the majority of studies addressing this issue that has led to the persisting uncertainties. Other possibilities include a direct effect of gonadal steroids on the central nervous system,[562] estrogen-induced production of subtypes of renin substrate with enhanced reactivity for renin,[563] increased concentrations of ethinylestradiol in hypertensive oral contraceptive users[564] or changes in catecholamine levels.[565]

4.5.4 Clinical Significance

Increased surveillance of BP is likely to have considerably diminished the number of women taking oral contraceptives who have hypertension. Nevertheless, since small increments in population BP means may increase morbidity even in the normal range of BPs,[566,567] it is important to consider what effect some of the lesser increases in BP seen in groups of oral contraceptive users might have. This issue was addressed in detail by Prentice.[568] Employed in this analysis were summary relative risk and odds ratio estimates from cohort and case-control studies of oral contraceptive users for subarachnoid haemorrhage, nonhaemorrhagic stroke and MI based on existing epidemiological studies.[569] It was assumed that oral contraceptive use is associated with average BP increases of 5.0mm Hg systolic and 2.0mm Hg diastolic. Relative risk functions relating unit rises in BP to incidence of each type of vascular disease in the age range 16 to 49 were derived from a Japanese cohort.[570] It was then estimated whether the rise in BP in oral contraceptive users was sufficient to account for the accompanying risks of vascular disease. It was noted that BP measurements possess appreciable measurement error so that the relative risk functions that relate disease incidence to measured BP may be much flatter than those relating to 'true' BP. A factor was therefore introduced into the analysis which took into account measurement error based on intra-individual variability.[571] The maximum estimated relative risks resulting from increases of 5mm Hg SBP and 2mm Hg DBP were 2.2 for subarachnoid haemorrhage, 1.7 for nonhaemorrhagic stroke and 1.2 for MI. Prentice[568] concluded that oral contraceptive-induced shifts in BP could explain haemorrhagic stroke, but only 10 to 20% of cerebral infarction and MI. The generalisability of the data on which these estimates were based may be criticised, but the conclusion appears to accord with what might be expected from the pathogenesis of these different conditions in oral contraceptive users.

4.5.5 Conclusions

There is little doubt that oral contraceptives can increase mean BP levels and that in certain susceptible individuals this increase qualifies them for a diagnosis of hypertension. It is also likely that increased BP has contributed to increased incidence

of subarachnoid haemorrhage in oral contraceptive users in some studies, although the contribution of increased BP to increased risk of myocardial and cerebral infarction is likely to have been less. Nevertheless, there is some evidence that oral contraceptive users who have received BP checks have a lower incidence of MI,[100] and increased attention to BP changes in women prescribed oral contraceptives is at least likely to have led to reduced incidence of hypertension in oral contraceptive users. However, considerable uncertainties remain regarding the relative importance of estrogen and progestogen components, the extent to which different progestogens differ in their effects, and the nature of the mechanism that underlies oral contraceptive-induced increases in BP. Reductions in oral contraceptive steroid dose and use of progestogens other than levonorgesterel may have had a beneficial effect on BP profiles, but, given the ease of the procedure, repeated monitoring in oral contraceptive users would seem the single most reliable safety measure with regard to BP.

4.6 Homocysteine

There is continuing interest in the role of homocysteine in vascular disease. Attention has tended to focus on atherosclerosis, but endothelial damage and increased risk of thrombosis appear to be the principal disturbances induced by elevated homocysteine levels. These issues have been reviewed in detail[572,573] and the importance of homocysteine as a vascular disease risk factor has recently been questioned.[574] The main concerns are summarised here, with particular emphasis on the potential importance of estrogen-induced disturbances in the vitamin cofactors of homocysteine metabolism.

Homocysteine is an intermediate in the metabolism of the sulphur-containing amino acid, methionine.[575] It has two alternative metabolic fates (fig. 20). One of these is conversion to cysteine and α-ketobutyrate by the transsulfuration pathway. Two pyridoxine (vitamin B_6)-dependent enzymes are involved in this conversion. The other metabolic path is for homocysteine to be converted back to methionine via the remethylation pathway. Methylcobalamin (vitamin B_{12}) and methyltetrahydrofolic acid (folic acid) serve as cofactor and cosubstrate, respectively, for this conversion. Which pathway is favoured depends on availability of methionine; transulfuration predominates when there is methionine excess and remethylation when there is methionine deficiency. In the fasting state in a normal population, homocysteine levels range between 5 and 15 μmol/L.[576,577] Levels higher than this constitute hyperhomocysteinaemia.

A number of factors can result in hyperhomocysteinaemia, the classic condition being an inherited deficiency in cystathione β-synthase, one of the vitamin B_6-dependent enzymes of the transsulfuration pathway. This results in highly elevated plasma homocysteine levels and the appearance of homocysteine in the urine, homocysteinuria. Other, rarer, enzyme deficiencies which cause highly elevated homocysteine levels involve methylenetetrahydrofolate reductase (MTHFR) and methionine synthase deficiencies. More moderate increases result from a common point mutation in the MTHFR gene, which reduces the enzyme's activity by about 50%,[578] is present in 10 to 13% of White populations, and renders carriers susceptible to the effects of suboptimal folic acid intake.[579] The most important determinant of general population variation in homocysteine levels, however, appears to be deficiency in the vitamin cofactor and cosubstrates involved in homocysteine metabolism. Both vitamin B_{12} and folic acid deficiency are associated with marked elevations in homocysteine levels and, in healthy individuals, negative correlations have been reported between homocysteine and vitamin B_{12} and folic acid levels.[577] Such correlations are also seen with vitamin B_6 although there is conflicting data as to whether vitamin B_6 deficiency causes hyperhomocysteinaemia. Other factors associated with high homocysteine levels include increased age, male gender and, in women, menopause. Cofactor deficiency may contribute to some of these associations. Impaired renal function is also associated with elevated plasma homocysteine levels, resulting in the positive association that

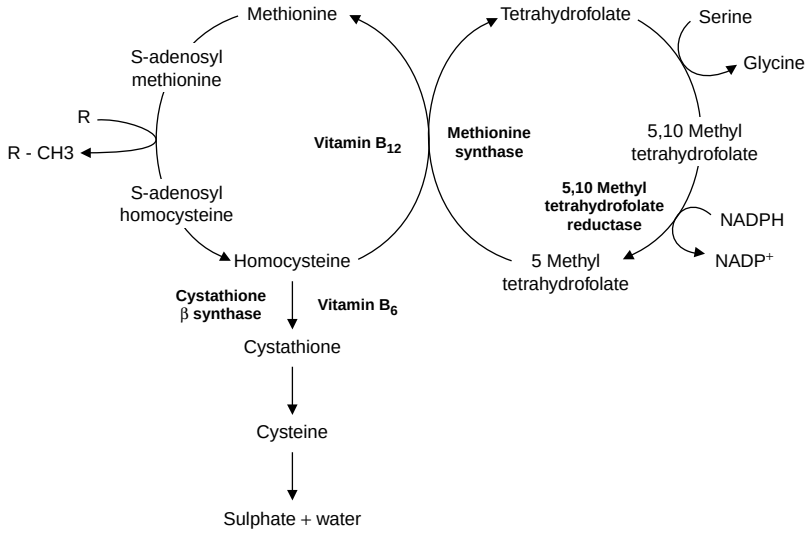

Fig. 20. Homocysteine metabolism. **NADPH** = nicotinamide adenine dinucleotide phosphate.

has been reported between homocysteine and creatinine levels.

Hyperhomocysteinaemia may be alleviated by folic acid supplementation (0.2 to 0.7 mg/day) and homocysteine levels rise when vitamin supplementation ceases.[580] In contrast to cosubstrate supplementation with folic acid, it would be expected that cofactor supplementation with vitamins B_6 or B_{12} (pyridoxine or cyanocobalamin) would only be beneficial when levels are suboptimal or frankly deficient. This has been the case in studies of vitamin B_{12} supplementation.[581] Administration of vitamin B_6 alone has not been found to lower homocysteine levels in healthy individuals. In patients with peripheral or cerebrovascular disease, folic acid combined with pyridoxine reduces homocysteine levels by 53%.[582] Pyridoxine alone had no effect but did lower homocysteine levels following a methionine load. In post-MI patients, various combinations of folic acid, vitamin B_6 and vitamin B_{12} have been used to lower homocysteine levels.[583] Folic acid was also effective when given alone.

4.6.1 Homocysteine and Cardiovascular Disease

The involvement of homocysteine in vascular disease is exemplified in patients with homocysteinuria. These patients are at significantly increased risk of both venous and arterial thrombosis and may exhibit lesions in any vascular bed. Marked intimal thickening and disruption of the internal elastic lamina are characteristic, and proliferation of perivascular tissues is also apparent.[584] Elevated homocysteine levels have been repeatedly demonstrated in patients with vascular disease.[585] Whereas the prevalence of hyperhomocysteinaemia in the general population appears to be about 5%, it has been estimated at between 13 and 46% among patients with atherosclerotic vascular disease. In a meta-analysis of 27 studies (including 23 cross-sectional or case-control studies), odds ratios of 1.7 (95% CI 1.5 to 1.9) for coronary, 2.5 (2.0 to 3.0) for cerebral and 6.8 (2.9 to 15.8) for peripheral atherosclerotic diseases were reported.[586] Meta-analyses of studies of associations between hyperhomocysteinaemia and venous thrombosis gave an odds ratio of 3.0 (2.1 to 4.2).[587] However, there have been inconsistent findings reported in prospective cohort studies, with relative

risks for coronary heart disease of between 0.8 and 4.5.[573] This leaves open the possibility that elevated homocysteine levels may be a consequence rather than a cause of coronary heart disease.[574]

As well as having higher homocysteine levels, patients with vascular disease may also have low levels of vitamins B_6 and B_{12} and folic acid.[588] It is noteworthy that vascular lesions have been seen to develop in animals chronically deprived of vitamin B_6.[589] Moreover, mutations in the MTHFR gene, which are associated with elevated homocysteine levels, are also associated with increased risk of vascular disease.[590]

Vascular occlusion associated with elevated homocysteine levels may result from disturbances at several levels, especially in the endothelium and the haemostatic system. Early *in vitro* studies showed that, in the presence of copper, homocysteine has a cytotoxic effect on endothelial cells.[591] Moreover, oxidative effects associated with exposure of endothelial cells to homocysteine have been detected.[592] Flow-mediated vasodilation is impaired in patients with homocysteinuria, although this is not seen in their heterozygote parents.[593] In this respect, the observation by Chambers et al.[594] that an acute elevation in homocysteine following an oral methionine load could significantly impair flow-mediated vasodilation – an endothelium-dependent process – in healthy humans is important. Mean homocysteine levels were increased from 9.3 to 30.5 μmol/L, the effect was not seen with placebo, neither was it seen when vasodilation was induced by nitroglycerin, a non-endothelial mediator. There is also the possibility that homocysteine may affect platelet function. Increased platelet adhesiveness has been described in response to homocysteine, as well as abnormalities in adenosine diphosphate-induced platelet aggregation. Increased thromboxane A_2 (TXA_2) formation has been reported in homocysteinuric patients.[595] Thus, homocysteine-induced changes in arachidonic acid metabolism could result in the accumulation of powerful platelet aggregators in association with vascular episodes, leading to a marked increase in thrombotic tendency.

Reduced antithrombin activity has been described in patients with homocysteinuria and promotion of procoagulant activity by homocysteine has been demonstrated in several studies. Prothrombin activation of factor X and activation of factor V are stimulated by homocysteine.[596] Moreover, homocysteine can inhibit both surface expression of thrombomodulin and protein C activation in cultured endothelial cells[597]

4.6.2 Homocysteine Levels in Oral Contraceptive Users

Administration of estrogens alone can lower homocysteine levels[598,599] and, in accord with this, women taking oral contraceptives usually have reduced or unchanged homocysteine levels.[600] However, some abnormally high responses have been reported.[601] That these elevated levels might be important was suggested by the study of Beaumont and colleagues[602] in which significantly elevated homocysteine levels were found in 100 oral contraceptive users who had experienced an occlusive vascular event compared with 100 oral contraceptive users who had remained healthy. The cases included 36 women with pulmonary or venous thrombosis, 6 with arterial thrombosis and 58 with cerebrovascular occlusion. Mean homocysteine levels in cases and controls, and in 10 women who had never used oral contraceptives are shown in figure 21. In both logistic and stepwise regression analyses, homocysteine levels emerged as an independent correlate of the presence of occlusive vascular disease.

The few reports of disturbances in homocysteine metabolism in oral contraceptive users show striking parallels with studies describing disturbed tryptophan metabolism in women taking oral contraceptives, in that there appears to be a distinct individual susceptibility.[603] Moreover, disturbances in tryptophan metabolism have been linked with deficiencies in oral contraceptive users in those vitamins that might also be expected to adversely affect homocysteine metabolism.[604] Disturbances in tryptophan metabolism in oral contraceptive users are known to arise from vitamin B_6 deficiency. This may be traced to the increase in

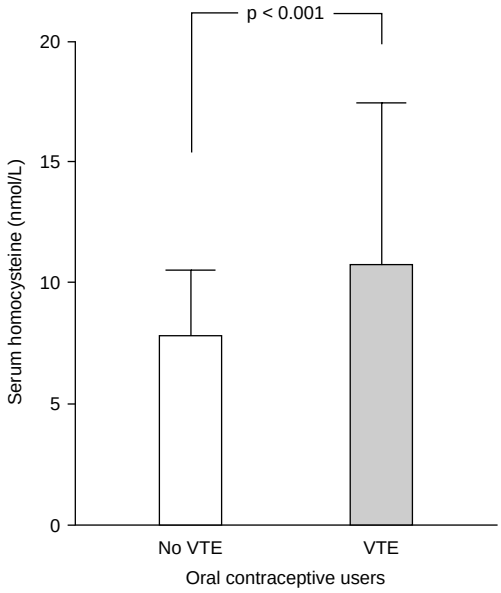

Fig. 21. Elevated homocysteine levels in oral contraceptive users who have had a venous thromboembolic event [VTE] (n = 100) compared with levels in oral contraceptive users who have not (n = 100).[602]

corticosteroid activity associated with the potent alkylated estrogens used in combined oral contraceptives. Increased corticosteroid activity then induces the principal enzyme in tryptophan metabolism, tryptophan pyrollase. Increased tryptophan metabolism causes a drain on one of the principal cofactors in tryptophan metabolism, vitamin B_6, and this results in diversion of tryptophan catabolism away from quinolinic acid. Quinolinic acid normally suppresses hepatic gluconeogenesis so reductions in quinolinic acid production result in increased hepatic gluconeogenesis and deterioration in glucose tolerance. In classic work undertaken by Rose, Adams and colleagues[603] in the early 1970s, it was shown that vitamin B_6 supplementation could reverse the deterioration in glucose tolerance associated with oral contraceptive use, but this reversible deterioration was only seen in women with a biochemically-detectable subclinical vitamin B_6 deficiency.

Therefore, there is the possibility of a convergence between findings with regard to tryptophan and homocysteine metabolism. As with impairment of glucose tolerance, hyperhomocysteinemia may only be seen in women with a subclinical vitamin B_6 deficiency. Moreover, such hyperhomocysteinaemia would be best detected by methionine loading, which does not appear to have been explored in oral contraceptive users. Other vitamin deficiencies that have been reported in oral contraceptive users include folic acid and vitamin B_{12} which would be expected to have a still more adverse effect on homocysteine metabolism.[605] Bearing in mind that endothelial damage by homocysteine is copper dependent, it is noteworthy that serum copper levels may be raised in women taking oral contraceptives.[605] Whether vitamin supplementation might reduce homocysteine levels in oral contraceptive users with hyperhomocysteinemia remains to be investigated. Given the prothrombotic changes associated with hyperhomocysteinemia, including increased resistance to APC and factor V activation, it would be of interest to investigate further the possible role of hyperhomocysteinemia in the increased risk of venous thrombosis in oral contraceptive users. Interactions with the genetic background will need to be elucidated in this respect, as suggested by the recent case report of pulmonary embolism during pregnancy in a young woman heterozygous for factor V Leiden mutation and homozygous for a MTHFR point mutation[606] Depending on the outcome of these investigations, it might be of interest to investigate the influence of vitamin supplementation on increased risk of VTE in oral contraceptive users.

4.7 Circulating Immune Complexes

The intriguing possibility that occlusive vascular disease in oral contraceptive users might be due to activation of the immune system was initiated by Beaumont and colleagues.[607-609] Such an activation was first suggested by the observation of antibodies to ethinylestradiol in an oral contraceptive user who had experienced a pulmonary embolism.[610] Isolation of circulating immune com-

plexes containing anti-ethinylestradiol antibodies, and their further characterisation and development of methods for their identification followed.

In a case-control study of women in the UK, it was found that women who had used oral contraceptives prior to experiencing a pulmonary embolism or idiopathic cerebral thrombosis tended to have higher levels of these immune complexes than did a group of apparently healthy controls,[611] independent of whether the controls were themselves taking oral contraceptives. Women who had used oral contraceptives prior to experiencing a MI, but did not have higher levels of circulating immune complexes, generally had other vascular disease risk factors. Circulating immune complexes therefore tended to be raised in oral contraceptive users who had experienced an occlusive vascular event which could not be explained by other risk factors, suggesting a specific association between oral contraceptive use, immune activation and vascular occlusion. It was observed that in most cases the isolated immune complexes had binding affinity for ethinylestradiol.[612] The associations were independent of age and duration of oral contraceptive use and no association was found between dose of ethinylestradiol and levels of circulating immune complexes.

Further confirmation of these observations has followed.[602,613,614] In an analysis of anti-ethinylestradiol antibody levels in 1318 oral contraceptive users, evaluated between 1976 and 1988, who had experienced an arterial or venous thrombotic event, Beaumont et al.[614] found that such antibodies were not present in any of the 61 women who had never used oral contraceptives, but were present in 33% of healthy oral contraceptive users (n = 124) and in 72% of those with thrombosis. Mean antibody levels were 5-times higher in oral contraceptive users who had experienced a thrombotic event compared with those who had remained healthy. Cases who did not have antibodies tended to have other risk factors for occlusive vascular disease. Little association was found between oral contraceptive formulation or duration of use and the presence of anti-ethinylestradiol antibodies, but in 47.7% of oral contraceptive users with thrombosis it was noted that antibodies and cigarette smoking were present together. In another study, comparing 100 healthy oral contraceptive users with 100 women who had experienced a vascular occlusive event while taking oral contraceptives, the most powerful independent discriminators of whether or not a woman had experienced an occlusive event were the presence of anti-ethinylestradiol antibodies and serum homocysteine levels.[602] Cigarette smoking and age were also independent discriminators, but were less powerful. These four variables could together achieve 66% accuracy in discriminating those who had and had not experienced a vascular occlusive event.

The pathophysiological significance of circulating anti-ethinylestradiol antibodies remains unclear. They could be indicative of an underlying vascular inflammatory condition, consequent on repeated endothelial damage, which then provides conditions under which there might be particularly vigorous anti-estrogen antibody formation. However, as described previously, histopathological studies of the vasculature in oral contraceptive users who have experienced a fatal vascular occlusion have found little evidence for inflammatory changes. Alternatively, the immune complexes which these antibodies form might damage the endothelium, promote intimal hyperplasia and interfere with the haemostatic balance at the endothelial level. However, it should be mentioned that one study has not confirmed the presence of anti-ethinylestradiol antibodies in oral contraceptive users who experienced a thrombotic stroke, and has questioned the methodology previously employed.[615] Clearly, this area requires further investigation if its true importance is to be accurately assessed.

4.8 Platelets, Peroxides and Prostaglandins

Platelet activation and aggregation, and the prothrombotic changes and vasoconstriction that can accompany these processes, have been mentioned in previous sections on vascular pathology. In oral contraceptive users, platelet activity *per se* has

been the focus of several studies, particularly in relation to changes in lipid peroxidation and prostaglandin (PG) metabolism, and these issues are summarised briefly here.

The first indication of altered platelet activity in oral contraceptive users emerged from studies of platelet electrophoretic mobility undertaken in the later part of the 1960s.[616] It was observed that platelet behaviour in women taking estrogen-containing (but not progestogen alone) oral contraceptives resembled that seen in patients with ischaemic heart disease; there was an increased sensitivity of the mobility of platelets to ADP, and platelet mobility was higher. These changes were ascribed to changes in phospholipid metabolism. Evidence followed that these characteristics could translate into a functional platelet hyperaggregability in oral contraceptive users.[617-619] In the majority of studies, this increase in platelet aggregability was seen in association with estrogen/progestogen combined oral contraceptives, but not progestogen-only formulations. There have been exceptions to the reported lack of effect of progestogens however; for example, in the cases of chlormadinone, cyproterone (cyproterone acetate) and subdermally implanted norgestrel.[620-622] In reviewing effects of progestogens on platelet activity, Kuhl[623] concluded that the greatest effects were seen with combined oral contraceptives containing more androgenic progestogens.

In a series of papers, Ciavatti and colleagues[624-628] identified increased polyunsaturated fatty acids, increased platelet lipid biosynthesis and increased formation of lipid peroxides as the principal factors involved in this increased aggregability. These effects could be overcome by vitamin E (tocopherol) supplementation in oral contraceptive users.[629] Oral contraceptive use was known to be associated with low vitamin E levels,[630] so the possibility emerged that there could be enhanced platelet aggregability in oral contraceptive users because of their depleted antioxidant status.

Since lipid peroxidation was known to be associated with reduced antithrombin III activity,[631] there was also the possibility of a convergence between these effects and the reduced antithrombin III activity seen in oral contraceptive users. Another point of convergence lay in modulation of platelet activity and aggregation by prostaglandins. Lipid peroxides could affect the balance between TXA_2 and prostacyclin (PGI_2) in favour of TXA_2 activity, and there was evidence, albeit conflicting, that oral contraceptives could enhance platelet aggregation in response to TXA_2 or reduce platelet responsiveness to the antiaggregatory effects of prostacyclin.[632-634] There was also evidence that oral contraceptives could independently affect prostaglandin metabolism. Ylikorkala and colleagues[635] found increased excretion of prostacyclin metabolites, an index of increased prostacyclin synthesis, in users of a low estrogen dose desogestrel-containing formulation but a slight decrease in users of an equivalent dose levonorgestrel containing formulation. There was no change when the progestogens were given alone. On the basis of work showing stimulation of prostacyclin synthesis by HDL, the authors had hypothesised that such differences might relate to the rise in HDL levels associated with use of the former combination, and fall in HDL with the latter. However, although pre-oral contraceptive treatment prostacyclin excretion correlated positively with HDL cholesterol levels, particularly in the HDL_2 subfraction, the changes in HDL and metabolite excretion accompanying treatment did not correlate.

Both prostacyclin metabolism and platelet aggregation were examined in a study of the effects of smoking in oral contraceptive users by Mileikowsky and colleagues.[636] Four groups of women were compared, divided according to whether or not they were smokers and whether or not they were taking oral contraceptives. Among oral contraceptive users, prostacyclin metabolite excretion was reduced in smokers, but only among those who had been smoking for 5 years or more. In response to inhalation of cigarette smoke, prostacyclin metabolite excretion was unaffected among those not taking oral contraceptives, but was significantly reduced among those taking oral contraceptives. This reduction in prostacyclin metabolite excre-

tion in oral contraceptive users in response to the acute effects of cigarette smoke was accompanied by a significant increase in platelet aggregation, particularly in response to a thromboxane analogue.

In summary, as with homocysteine metabolism, there is an intriguing convergence between adverse changes in a vascular risk factor in oral contraceptive users and low vitamin levels, in this case with regard to platelet aggregability and the antioxidant vitamin E. This is particularly interesting because of the significant role of cigarette smoking in enhancing platelet aggregation in oral contraceptive users. The association of these factors recalls the particular characteristics of arterial disease in oral contraceptive users described in sections 2 and 3. These included (i) angiography suggestive of a transient occlusion, which, in an artery, could be most easily explained by platelet deposition, and (ii) the strong synergistic effects of oral contraceptive use and cigarette smoking in promoting such arterial occlusions. It is noteworthy that in a recent study of antioxidant status in oral contraceptive users, there was a marked deterioration in serum β-carotene levels with increasing age.[637] It is possible that the surprisingly negative findings in two recent analyses of oral contraceptive use, cigarette smoking and MI,[120,121] might reflect the beneficial influence of increased antioxidant intake.

4.9 The Haemostatic System

As described in section 3.1, the vascular endothelium is critical to haemostatic function, and a broad range of factors are elaborated by and interact with the endothelium to affect haemostasis. Each of the factors described in this section could provide an index of thrombotic risk, vascular dysfunction or risk of arterial occlusion. The extent to which each factor can provide this information and the ways in which these factors are affected by oral contraceptives will be considered in detail after the following description of the functioning of the system as a whole. A simplified overview of the haemostatic system is presented in figure 22.

4.9.1 Haemostatic Function

The Coagulation Cascade and the Vascular Endothelium

In addition to its antithrombotic actions, the vascular endothelium has a critical role in the mediation of processes that prevent blood loss from damaged vessels. Endothelial damage and consequent exposure of subendothelial tissue results in the predominance of vasoconstrictor responses, tissue factor release and adhesion of activated platelets. These processes culminate in fibrin generation (coagulation) and fibrin deposition (clotting). It has become increasingly appreciated that these processes occur against the background of a tonic low-grade activation of the haemostatic system, even in the absence of overt vascular disease.[638] Moreover, intravascular fibrin formation may be a cause as well as a symptom of endothelial dysfunction. Consequently, it may be difficult to distinguish whether fibrin formation is a cause or effect of endothelial damage.

Endothelial Damage

Endothelial expression of tissue factor, a membrane protein not normally exposed to the circulation, initiates clotting at the site of endothelial damage. According to the cascade model of coagulation, the initial binding of tissue factor to activated FVII (FVIIa) causes rapid autocatalytic generation of further FVIIa, which in turn activates several other coagulation factors.[639] Activation of haemostatic factors involves the generation of active proteases (which are designated by the suffix a) from their inactive zymogens. Activation of factors X and V requires the assembly of activated factors on activated membranes, which may be provided by platelets,[640] leucocytes[641,642] or endothelial cells.[643] The resulting platelet plug forms a first barrier against blood loss from vascular injury and also promotes localised amplification of the clotting cascade, the final step of which is the cleavage of the coagulatory protease, thrombin, from its inactive zymogen, prothrombin. Thrombin then rapidly generates fibrin monomers from circulating fibrinogen by proteolysis, generating clots

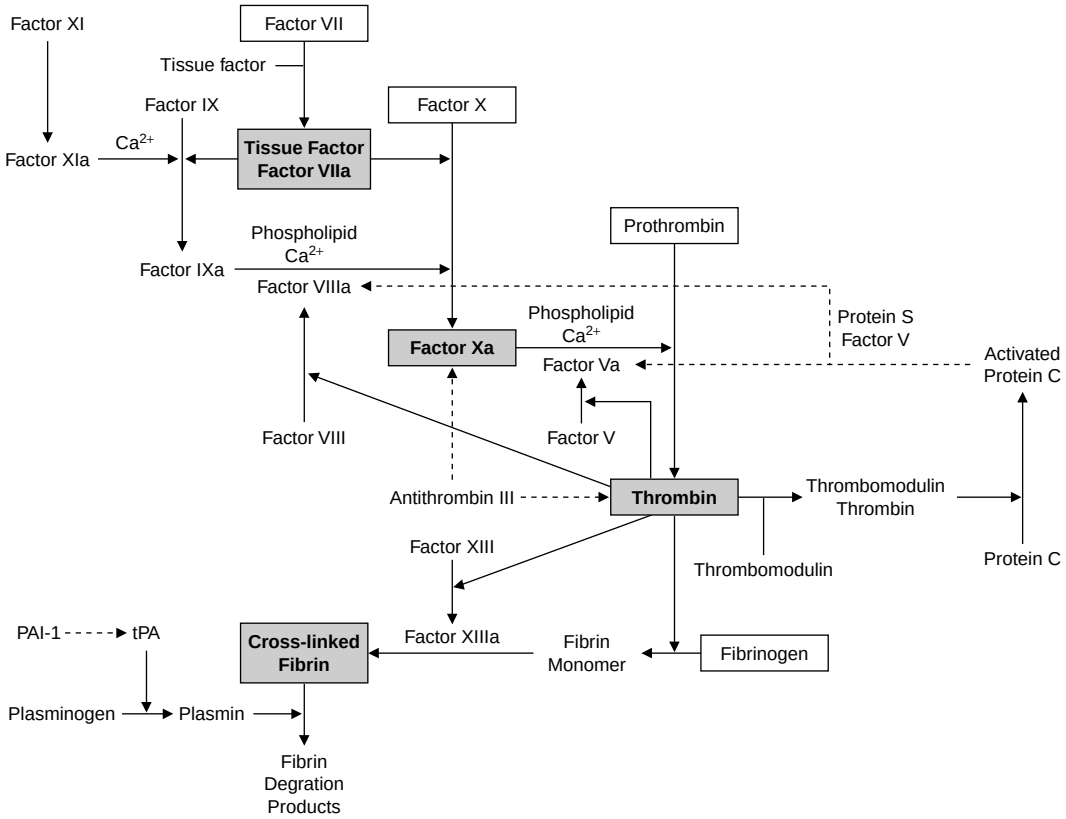

Fig. 22. Factors of the haemostatic system and their interactions. Broken lines indicate inhibition/inactivation. **PAI-1** = plasminogen activator inhibitor-1; **tPA** = tissue plasminogen activator.

composed of platelets and cross-linked fibrin polymers at the site of endothelial damage.

Regulation of the Coagulation Cascade

Feedback controls are exerted on the haemostatic system to an extent that depends on the activity of the system itself. Extrinsic activation of the coagulation cascade, via tissue factor and FVII, amplifies the procoagulatory signal via the intrinsic pathway of coagulation.[644] Trace amounts of thrombin can activate the intrinsic pathway via factor XI, and this appears to account for the unique 'signal-intensifying' feed-back activation within the coagulatory cascade.[645]

In addition to feed-back activation within the plasma, the proteolytic reactions of the coagulation cascade are also regulated at the lumenal surface of the endothelium. Many membrane-bound proteins act as cofactors or receptors. Their expression provides an important mechanism in the control of coagulation activity and in ensuring that coagulation is restricted to the site of endothelial damage.

Two major anticoagulant pathways have been identified: antithrombin III (AT III), and the protein C and S system. The anticoagulatory activity of protein C requires the expression of an endothelial protein, thrombomodulin, which stabilises the conversion of protein C to APC.[646] In the presence of free protein S, APC inhibits coagulation by proteolytic cleavage of activated factors VIII and V. Thus, APC acts mainly on the feed-back activation of the intrinsic pathway of coagulation. The AT III pathway inhibits several activated coagula-

tion enzymes, mainly of the extrinsic pathway, by forming inactive complexes such as the thrombin-AT III complex. These are rapidly cleared from the circulation by the reticuloendothelial system.

The basal activity of both the AT III and protein C/protein S pathways is low, and activation at the endothelial surface is required for the pathways to fully operate. The activated endothelium plays an important role in these regulatory mechanisms by expressing membrane-bound cofactors, including thrombomodulin and heparin-like glycoproteins, which accelerate thrombin inhibition by these pathways. Thrombosis may result from either a deficiency in the plasma reservoir of coagulation inhibitors or from dysfunction of the endothelial activating mechanisms. It is important to note that insufficient anticoagulatory capacity may be present despite normal plasma levels of inhibitors because of inadequate expression of endothelial cofactors.

The Fibrinolytic System

Endothelial control of fibrin deposition is also exerted via the fibrinolytic system.[647] Thus, little baseline fibrinolytic activity may be expected as long as there is no detectable clotting activity.[648] However, there is a tonic low grade activation of the fibrinolytic system comparable with the continuous low grade activity of coagulation, in both cases most probably induced by the same endothelial activity.[649] In response to the same stimuli which initiate coagulation, the endothelium can itself release tissue plasminogen activator (t-PA), and its rapid inhibitor, PAI-1. Mechanisms regulating the balance between plasminogen activation and inhibition are poorly understood.[650] The most powerful stimulus to plasmin generation is the fibrin clot itself to which plasminogen must bind to be adequately presented to t-PA. The fibrinolytic response to clot formation may be appreciably inhibited by competition at the binding site by factors such as histidine rich glycoprotein (HRG)[651] and Lp(a).

Plasmin splits fibrin into fibrin degradation products (FDPs). *In vivo*, the half-life of plasmin is measured in milliseconds. Plasmin is inactivated by several inhibitors, the most abundant and active being α2 antiplasmin.[652] The end-product of this inhibition, the plasmin-antiplasmin complex (PAP) has a half-life in the range of 30 minutes. Assessments of PAP in addition to FDPs may therefore serve to reflect fibrinolytic activity.

4.9.2 Evaluation of the Haemostatic System

Blood collection and handling is a major source of bias and error in haemostatic analyses. *Ex vivo* 'contact' activation of the coagulation cascade cannot be completely avoided but may be minimised by standardised blood sampling and handling protocols. Traditionally, coagulation factors were determined by clotting assays using factor-deficient plasma to titrate the 'percentage of the normal value' of the sample. These units are still in use, although new technologies allow for a direct assessment of protein concentrations and enzymatic activities. However, protein concentrations provide only limited information on the functional availability of a molecule. The effects of inhibitors or cofactor deficiencies cannot be assessed by an immunological measurement and may require assessment of *in vivo* activity. However, most activity assays measure *ex vivo* activities, thus measuring what may more accurately be referred to as 'activatable capacity'.[653]

Likewise, it has been questioned whether any study of the fibrinolytic system under steady-state conditions provides meaningful information. Since the fibrinolytic system is operating on demand, the activity of the system is likely to depend largely on the coagulatory input. Therefore, a standardised challenge such as 10 minutes venous occlusion or stimulation with a vasopressin analogue (desmopressin) has been proposed for assessment of activatable fibrinolytic activity or fibrinolytic capacity.[654]

Reaction products of the various haemostatic enzymes may provide the best estimate of *in vivo* activity. However, precision of assessments of these markers is largely affected by quality of blood collection and handling. A certain *ex vivo* activation during the procedure cannot be avoided, but the extent to which this will affect the overall assess-

ment depends mainly on the degree of *in vivo* activation present, i.e. the precision of reaction markers is higher if their *in vivo* concentrations are high. Therefore, markers such as fibrinopeptide-a and thrombin-antithrombin complex (TAT) with half-lives in the range of less then 10 minutes cannot be determined as precisely as markers with half-lives of 30 minutes and more, such as prothrombin fragment 1+2 (F_{1+2}), FDP or PAP.

4.9.3 Haemostatic Variables and Vascular Disease

Venous Thrombosis

Progress in understanding the relationship between VTE and disturbances in the haemostatic system has been impeded by the extraordinary interdependence that exists between the various pro- and anti-thrombotic and pro- and anti-fibrinolytic factors. The only disturbances that have been unequivocally linked to the development of venous thrombosis relate to the inherited thrombophilias, in which major deficiencies in plasma levels of anticoagulatory factors are seen. Based on this model it has become widely accepted that mechanisms associated with low anticoagulatory capacity or increased coagulation workload should be considered as potentially thrombogenic.[638,643,655] It may therefore be appropriate to consider thrombosis as resulting from congenital or acquired thrombophilias, or a combination of both (fig. 23). A third approach emerging from case-control and observational studies has focused on the acute phase reaction that is known to be a powerful stimulator of coagulation in cytokine mediated inflammatory diseases such as endotoxaemia.

Decreased Anticoagulatory or Fibrinolytic Capacity

There is convincing evidence linking congenital thrombin-inhibitor deficiencies with venous thrombosis.[657] AT III, protein C and protein S deficiencies are unlikely, if plasma activities are within the range of normal variation (80 to 120% of normal). However, this does not imply that values below the normal range increase risk. In fact, the clinical significance of environmental changes of these inhibitors are difficult to interpret.[658] There is evidence that, in thrombophilia, venous

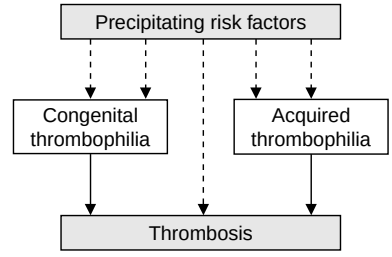

Fig. 23. Thrombotic disease may be precipitated by 'trigger' conditions, particularly in individuals with congenital or acquired predispositions ('prethrombotic state').[656]

thrombotic risk is linked with the thrombophilic genotype rather than with the phenotypic variation in the absolute plasma levels of the inhibitors. In the Leiden Thrombophilia database, the association of risk with variation in genotype of AT III or protein C deficiency was twice as great as with the phenotypic variation.[659] Moreover, prospective studies in affected families have provided convincing evidence that the manifestation of thrombotic disease in the individual patient is frequently linked with precipitating situations such as surgery, immobilisation and the puerperium.[660] These observations suggest that the thrombophilic trait creates a latent, prethrombotic state[655] which may be thrombogenic only when the anticoagulatory capacity is further compromised or the coagulation workload is critically increased.[657,661] Recently, the significance of cofactors in anticoagulatory system function has been highlighted by the study of APC resistance. In view of the importance that has recently been ascribed to APC resistance in oral contraceptive-induced thrombosis, the next subsection has been devoted to fundamental issues in APC resistance.

In general, increased clot lysing activity counters procoagulant changes in healthy individuals. Conversely, insufficient fibrinolytic capacity may promote coagulation. Reduced fibrinolytic capacity due to high PAI-1 levels was found preoperatively in patients who subsequently developed thromboembolic disease after hip surgery.[662] The risk at-

tributable to a hypofibrinolytic state appears to be particularly high in the presence of pre-existing vascular disease and high coagulatory activity.[663] For instance, high PAI-1 levels predicted recurrence after VTE[664] and MI.[665] There is evidence of direct interrelations of the fibrinolytic system via PAI-1 with triglycerides and insulin metabolism, suggesting that the adverse effects of complex interrelated syndromes such as the insulin resistance syndrome may involve a compromise of clot lysing capacities via increased levels of PAI-1.[666,667]

Activated Protein C Resistance

The significance of cofactors in anticoagulatory system function has recently been highlighted by the study of APC resistance, and this has subsequently assumed considerable importance in the study of oral contraceptives and VTE. Associations between oral contraceptive use and APC resistance are mentioned in the present section as appropriate, but are dealt with in detail in subsequent sections on effects of oral contraceptives (section 4.9.4) and, the effects of different progestogens (section 5.7) on the haemostatic system.

In 1992, Dahlbäck and colleagues[668] described a patient who had experienced a venous thrombosis and who had a strong family history of venous thrombosis, but in whom there were none of the known defects in the haemostatic system. However, in this patient it was found that addition of activated protein C to plasma did not result in the expected prolongation of the activated partial thromboplastin time (aPTT). Addition of normal plasma to proband's plasma increased the prolongation of the aPTT by APC and it was concluded that the APC resistance could be best explained by a deficiency in an unidentified cofactor for protein C.

This APC resistance was subsequently confirmed in a number of case-control studies of venous thrombosis[669-672] and was found to be remarkably common in patients with venous thrombosis, being present in between 21 and 64% of those studied.[670-672] APC sensitivity was generally expressed in terms of the APC sensitivity ratio (APC-sr) i.e. the ratio between the aPTT with and without APC. APC resistance was considered to be present if the APC-sr was found to be below a certain lower limit defined statistically or with reference to the lowest value encountered in a control group.[670,671] APC resistance was found to confer a 7-fold increase in risk of DVT,[672] was inherited as an autosomal dominant trait, and displayed a distinctly bimodal distribution.[671,672]

The activity in plasma that could alleviate APC resistance was found to behave exactly like factor V.[673] On the basis of reconstitution experiments using factor V isolated from normal and APC resistant plasmas, and from studies of the inactivation by APC of factor V from normal and APC resistant plasmas, it was subsequently established that APC resistance was caused by a structural abnormality in factor V that rendered it resistant to inactivation by APC.[674]

The genetic basis for the phenotype of inherited APC resistance was first reported in 1994 by the Leiden Thrombophilia Study group.[675] APC resistance was associated with a point mutation G to A substitution at nucleotide position 1691 in the factor V gene, which had the effect of altering amino acid residue 506 from an arginine to a glutamine in the factor V molecule. Since this is the cleavage site for APC, the so-called factor V R506Q or factor V Leiden mutation determines the synthesis of a factor V molecule that cannot be effectively inactivated by APC. This mutation was present at a frequency that was at least 10-fold higher than that of all other known genetic risk factors for thrombosis in the Dutch population in which it was identified.

That the mutation in the factor V gene was the principal cause of APC resistance was confirmed by three other groups.[674,676-678] Those homozygous for the mutation had an 80-fold increase in risk of thrombosis, in contrast to the 7-fold increase seen in heterozygotes.[70] Dahlbäck's group noted that the mutation showed perfect co-segregation with the APC resistance phenotype, and could explain the great majority of cases (47 out of 50 studied) of inherited APC resistance.[679] The mutation was found to be relatively common among people of European origin, being present in about 4% of the population, thus making it an important risk

factor for venous thrombosis.[680] Moreover, regional variations were subsequently found, with a prevalence of up to 14% in some areas.[681]

Thrombosis could occur in carriers of the factor V Leiden mutation in the absence of other predisposing factors.[70] However, in case-control studies, risk of venous thrombosis in those carrying the factor V Leiden mutation, or with a degree of APC resistance consistent with the presence of the mutation, was considerably augmented by the presence of other risk factors.[671,679,682] These included trauma, surgery, pregnancy and, in particular, oral contraceptive use.[35,69]

The possibility that other risk factors for venous thrombosis might act in concert with factor V Leiden mutation by further lowering the APC sensitivity ratio was suggested by the observation that heterozygous family members who had experienced a thrombotic episode had lower APC sensitivity ratios than heterozygotes who had not.[679] Moreover, APC resistance was observed in the absence of the factor V Leiden mutation.[683] These observations suggested that beyond APC resistance inherited with the factor V Leiden mutation, there might be an acquired APC resistance. Characteristics found to be associated with a lower APC-sr independent of the factor V Leiden mutation were female gender,[672,684] pregnancy[685-688] and use of oral contraceptives.[684,689-691] In comparison with men, women had a 10% lower APC sensitivity ratio.[684,689,691] Pregnancy reduced the ratio by 15 to 32%[685-687] and oral contraceptive use by 4 to 8%.[684,689-691] The association between increased levels of female sex steroids and acquired APC resistance, drew attention to the possible influence of steroid-induced changes in other factors of the haemostatic system on the APC sensitivity ratio. In pregnancy, decreased free protein C levels correlated significantly with decreasing APC sensitivity,[687] and, in several studies, a lowered APC-sensitivity ratio was related to increased factor VIII-clotting activity.[685-688]

The great majority of studies of APC resistance have employed the APC sensitivity ratio as the measure of choice. In some studies, the activated protein C sensitivity ratio was normalised according to the APC sensitivity ratio in a normal pooled plasma (n-APC-sr). This was intended to promote the development of reliable diagnostic criteria for APC resistance by minimising the influence of intra- and inter-laboratory variation in batches of reagents, or in the calcium or APC concentrations used in different assays. The search for reliable diagnostic criteria and for measures of APC-sr that could reliably discriminate those with the factor V Leiden mutation motivated the introduction of a number of variant methods for measuring the APC-sr. Methods generally depended on evaluation of the influence of APC on a system in which fibrin generation could be measured. In the great majority of studies this system has involved measurement of the aPTT. However, several other approaches have been explored, including prothrombin time (PT)- and factor Xa-based assays.[669] Tripodi et al.[692] evaluated 13 different approaches to distinguishing the presence of the factor V Leiden mutation using measurement of APC resistance, with fibrin formation triggered via either the intrinsic or common pathways of coagulation. They concluded that aPTT-based methods were highly variable in their capacity to differentiate carriers from non-carriers, but that the sensitivity could be increased dramatically by predilution of samples with factor V-deficient plasma. The best discrimination was obtained by methods in which fibrin formation was triggered by addition of activated factor X or Russell viper venom, and it was presumed that this was because they were less influenced by variation in haemostatic factors that act upstream of the common pathway. aPTT- and PT-based assays correlated well ($r = 0.82$).[669] De Ronde and Bertina[683] observed that the APC sensitivity ratio measured using an aPTT-based system was sensitive to variation in levels of protein S and factors II, VIII, IX and X, but not factor V. However, variation in APC sensitivity ratio with coagulation factor activity appeared to become relatively small at levels around 100% of normal (although the effects of supra-normal levels were not explored). Comparative studies showed that aPTT-based assays were

sensitive to factor XII deficiency (overestimation of response to APC) and an increase in factor VIII [FVIII] (underestimation of response), whereas the PT-based assay was sensitive to FVII deficiency (overestimation of response)[685]. Use of an APC ratio measure eliminated the variation associated with factor XII deficiency and diminished the variation associated with increased FVIII levels, but did not diminish the difference associated with FVII deficiency using the PT-based assay. These studies established that the different methods for evaluating APC resistance could be differentially affected by variations in factors of the intrinsic and extrinsic coagulation pathways. Use of a ratio measure eliminated some of this extraneous variation but not that associated with FVII deficiency.

Direct measurement of thrombin rather than fibrin generation was a feature of several novel assays for APC resistance, which exploited measurement of the so-called endogenous thrombin potential (ETP). This was a development of the thrombin generation curve, 'one of the oldest tools of the coagulation trade',[693] which results from the combined activity of the prothrombin activating enzyme complex (prothrombinase) and the thrombin-inactivating processes i.e. the binding of thrombin by antithrombins such as AT III and α2-macroglobulin. Depending on the method used to estimate thrombin, the thrombin generation curve will either return to zero, if the clotting of fibrinogen is used, or a level somewhat above zero if a chromgenic substrate is used. The latter follows from the fact that the α2-macroglobulin/thrombin complex (variously referred to as the α2macroglobulin/thrombin complex or the α2M-thrombin complex) has a persistent amidolytic, and therefore chromogenic, activity.[693] The ETP was then the integrated area under the thrombin generation curve. The ETP value for a particular plasma sample was shown to be directly proportional to residual levels of amidolytic activity in a thrombin generation assay due to the α2M-IIa complex.[694] Measurement of residual amidolytic activity could then be used to as a functional assay to assess the combined effect of all the factors that may influence thrombin generation in a given sample.[695] The ETP represented the thrombin-forming capacity of a nontriggered system (in contrast to activation measures such as F_{1+2}).

Measurement of the ETP in this way was employed in an alternative approach to evaluating APC resistance by Duchemin et al.[696] These researchers used a thrombin generation assay system, triggered by addition of tissue factor and phospholipid (rabbit brain thromboplastin), which enabled endogenous activation of protein C by addition of thrombomodulin. The approach obviated the need for protein C deficient plasma or APC to evaluate APC resistance and had the advantage of also being sensitive to variation in protein C activation. The difference in ETP without and with thrombomodulin gave a measure of the inhibition of thrombin generation due to the activation and activity of protein C. To standardise for the effect of variation in α2 macroglobulin and other haemostatic factors, this difference was expressed as a percentage of the ETP without thrombomodulin. The percentage inhibition of thrombin generation was 49.8 in 95 women compared with 53.9 in 97 men, and 40.7 in 10 oral contraceptive users compared with 51.0 in 19 non-users.

Another approach to quantifying the ability of APC to modify the ETP was introduced by Rosing et al.[697] In this assay, thrombin generation was again triggered via the extrinsic pathway by addition to defibrinated plasma of phospholipid vesicles then tissue factor and calcium chloride. APC sensitivity was expressed as the ratio of ETP in the presence and absence of APC divided by the ratio determined in a normal plasma pool (with this measure, the ratio increased with decreasing APC sensitivity). This assay did not provide complete discrimination between women who were carriers or non-carriers of the factor V Leiden mutation but, in contrast to the aPTT-based assays, there was a marked discrimination between different oral contraceptives in their effects on the n-APC-sr (see section 5.7.1). These differences were independent of variation in protein S and were apparent in

women within 3 days of commencing oral contraceptive use.

Use of ETP as a measure of the effect of APC on the coagulation system, was evaluated with initiation of thrombin generation via either the intrinsic or extrinsic pathway by Nicolaes et al.[698] In healthy individuals there was wide variation in estimates of APC-sr using the intrinsically triggered pathway, so APC resistance was studied using the extrinsic system. Using the extrinsic pathway (EP)/ETP-derived n-APC-sr, there was complete discrimination between normals and those heterozygous for the factor V Leiden mutation. An abnormal n-APC-sr was also found in pregnancy, oral contraceptive use, anticoagulant therapy, protein S deficiency or in the presence of lupus anticoagulant. However, the confounding effects of these variations could be corrected by performing the assay on plasma diluted 10-fold in factor V deficient plasma.

The association between the factor V Leiden mutation and APC resistance established the principle that APC resistance could provide an index of thrombotic risk. The question then arose whether the different measures of APC sensitivity returned by the different assays described above differed in their associations with clinical thrombosis.

Associations between n-APC-sr, measured using the EP/ETP assay, and VTE were evaluated by Heinemann and colleagues[699] in a case-control study of 67 women who had experienced a thrombotic episode. APC sensitivity (measured at least 6 months after the event) in patients was compared with 290 age-matched population controls. The odds ratio for those with an elevated n-APC-sr, not carrying factor V Leiden mutation, was 0.56 (CI 0.26 to 1.22: 8 exposed cases, 45 non-exposed; 72 exposed controls, 200 non-exposed). When the analysis was limited to those not carrying factor V Leiden mutation and not currently taking oral contraceptives, the odds ratio was 0.97 (95% CI 0.43 to 2.20: 7 exposed cases, 42 non-exposed; 26 exposed controls, 154 non-exposed). It appears that among the cases, there were only 4 current oral contraceptive users, so numbers were too small to allow any inference about links between thrombosis and oral contraceptive-induced changes in n-APC-sr. However, this study was consistent with there being no association between thrombosis risk and post-thrombotic, post-acute phase variation in n-APC-sr measured by the EP/ETP method.

A similar study was carried out as part of the Leiden Thrombophilia Study.[700] This analysis was restricted to men, 'to exclude the effects of oral contraceptives'. The n-APC-sr was measured using the EP/ETP-based assay in 172 cases and 201 controls. After exclusion of factor V Leiden positive individuals, there was an odds ratio for DVT of 3.4 (95% CI 1.1 to 10.8) in the highest quartile (9 cases and 5 controls) of sensitivity ratio compared with the lowest quintile (47 cases and 89 controls). In contrast to the findings of Heinemann at al.,[699] this study accorded with there being an association between thrombosis risk and post-thrombotic, post-acute phase variation in n-APC-sr measured by the EP/ETP method.

Of possible relevance to the clinical significance of changes in the APC sensitivity ratio detected by the EP/ETP-based assay is a case-report of a woman, being monitored during her pregnancy for suspected thrombosis, who showed a 3-fold increase in the APC sensitivity ratio immediately postpartum and who experienced a superior sagittal sinus thrombosis 5 days later.[701]

Risk of VTE associated with acquired variation in the APC sensitivity ratio derived using an aPTT-based method was evaluated in the context of the Leiden Thrombophilia Study.[702] APC sensitivity ratios were compared in 337 first cases of DVT and 455 age- and sex-matched controls, with exclusion of all individuals who were factor V Leiden mutation positive or were receiving anticoagulant therapy. Among the cases, normalised APC sensitivity ratios were measured on average 15 months after the thrombotic episode (range 6 to 44). An APC sensitivity in the lowest quartile conferred an odds ratio of 4.4 (95% CI 2.9 to 6.60) for VTE compared with those in the highest quartile. There was an 11-fold increase in risk among those with a normalised APC sensitivity ratio of less than 0.89.

Adjustment for variation in age, sex, oral contraceptive use at the time of venepuncture, fibrinogen, factor II and X, and proteins C and S made little difference to the odds ratio. Adjustment for variation in age, sex and FVIII coagulant activity decreased the odds ratio to 2.5 (2.9 to 6.6), indicating that some but not all of the risk associated with variation in APC sensitivity ratio could be ascribed to variation in FVIII. The lack of effect of adjustment for oral contraceptive use is noteworthy since it might be taken to imply that oral contraceptive-induced changes in normalised APC sensitivity ratios are irrelevant to risk of venous thrombosis. However, only about 30 of the 337 cases were taking oral contraceptives at the time of venepuncture.[703]

Increased Coagulatory Workload

Tonic low-grade coagulation activity[704] as indicated by F_{1+2}, fibrinopeptide-a or TAT levels may reflect the sensitivity of the endothelium to activating stimuli such as inflammation, angiogenesis and tissue remodeling. Only the pattern of associated changes is suggestive with regard to the underlying mechanisms. An increase of FVIIc suggests endothelial damage with expression of tissue factor, while an increase of fibrinogen and PAI-1 suggests an underlying inflammatory, acute phase reaction. Coagulatory activity is frequently increased in asymptomatic carriers of an inherited thrombophilia.[638,705,706] Several conditions associated with an increased risk of venous thrombosis are characterised by increased activity of the coagulatory system. These include age,[707,708] cancer,[709-713] chemotherapy,[714] systemic lupus erythematosus,[715] the postoperative period after surgery,[716] and the period after a thromboembolic event.[717] The coagulation activity may proceed to the generation and cross-linking of fibrin. In such cases, adequate anticoagulant capacity and fibrinolytic activity are needed to prevent the formation and progression of microclots.[653] Independent of the underlying stimulating mechanisms, VTE may result from a local overload of coagulatory action if the local capacity of antithrombotic mechanisms (either anticoagulatory or fibrinolytic) is exceeded.

It is important to recognise that there is no established relationship between the level of coagulation activation and risk of thrombotic disease, particularly in healthy individuals. Changes in coagulation activity must be interpreted in the context of the availability of an adequate control capacity. The particularly high level of both baseline coagulation and fibrinolysis in centenarians is convincing evidence that cardiovascular health may be maintained despite high levels of baseline 'hypercoagulability'.[708] Nonetheless, given the many limitations of haemostatic analyses, it is important to note that any endothelial activation is likely to be reflected in an increase of activation markers. Since we do not know all possible pathogenetic mechanisms nor the appropiate methods to monitor them, activation markers are potentially the most sensitive tests to assess the state of endothelial function.

Acute Phase Reaction

A novel approach to the assessment of risk markers was recently introduced by the Leiden group. In their population-based case-control study, haemostatic factors of interest were studied in patients (at about 15 months after the thrombotic event) and matched controls.[672] This study provided evidence that high fibrinogen and FVIII levels (as well as low AT III and protein C activities) increase the risk of venous thrombotic disease, suggesting that these acute phase reactants might indicate a thrombogenic activation of the vessel wall.[718] Interestingly, FVII, although a key factor of the extrinsic coagulation cascade, was not associated with venous risk.[719] The Leiden Thrombophilia Study is the first case-control study that has given credit to *post hoc* bias, i.e. the bias that may result from the fact that the haemostatic system of cases was studied after the event and may therefore be affected by endothelial sequelae of disease. This bias tends to overestimate the role of risk factors such as FVIII and fibrinogen. In view of *post-hoc* bias, the validity of the observed lack of association of thrombosis with FVIIc and protein S was actually enhanced. The Leiden Thrombophilia Study overcame this potential *post hoc* bias, by comparing the

prevalence of genotypes associated with increased factor levels between patients and controls. DNA polymorphisms associated with low fibrinogen levels were significantly less frequent in cases than controls, but neither FVII levels nor the MSP I polymorphism associated with high FVII levels were over-represented in cases.

A general problem in the interpretation of markers of haemostatic system activity is the fact that data from one study population cannot be transferred easily into a different population. The methodology of the haemostatic assessments varies considerably among centres, so that comparisons based on absolute values must be highly speculative. More importantly, the study population for any retrospective or prospective studies is highly selected, yet must also provide for a reasonable study size. For instance, if patients undergoing major surgery were studied for markers of postoperative complications, one has to bear in mind the fact that patients may carry a considerable risk of vascular disease due to their underlying disease, as in the case of a malignancy. It is questionable whether the findings from such studies may be transferred to the general population, particularly in young women using oral contraceptives.

Arterial Thrombosis

There is considerable uncertainty over whether the model of venous thrombosis may also apply to the arterial system. There are obvious discrepancies: FVIIc has not been linked with venous thrombotic disease, but has been linked with arterial disease in some studies.[719,720] As described in section 2, cigarette smoking is a powerful risk factor for arterial occlusion but does not appear to influence venous occlusion, and congenital coagulation inhibitor deficiencies are at best only weak markers of coronary artery disease. A problem in drawing parallels between arterial and venous risk is that potential markers of arterial haemostatic activity need to pass through the microcirculation before being detected and quantified in venous blood samples. Nonetheless, several haemostatic markers have been linked with subsequent atherosclerotic disease (fibrinogen, FVII), extent of arterial thrombosis (F_{1+2}, FDPs), or the prognosis of arterial thrombosis (FVIIc, PAI-1).

Acute Phase Reactants

Up to 50% of the variance in plasma fibrinogen levels is related to genetic polymorphisms.[721] About 10% of the total variance can be attributed to environmental factors, but these may account particularly for the high and very high values. Fibrinogen levels are increased in conditions such as hyperlipidaemia, diabetes mellitus, obesity and hypertension, and with smoking, age, menopause and stress.[722,723] A 'chronic' acute phase reaction has been postulated as a common denominator for these environmental influences.[724] Hepatic fibrinogen synthesis is increased within hours in response to interleukin (IL)-6, a critical mediator of the acute phase reaction *in vivo*.[725] High fibrinogen levels were shown to favour endothelial fibrin deposition both by activation of platelets and haemorrheologic effects.

In prospective studies, fibrinogen level predicts both fatal and nonfatal stroke and MI in men.[720,726-728] The Framingham study suggested a similar association in women up to the age of 70 years.[729] Although fibrinogen emerged from these studies as an independent risk marker for cardiovascular disease, it is currently – in the absence of interventional studies – difficult to differentiate whether the association is attributable to the underlying acute phase reaction or to the increased fibrinogen level *per se*.

Attenuation of fibrinolytic activity is another important feature of the acute phase reaction and has been shown to be the most powerful predictor of sudden death in patients with stable angina pectoris.[730] Among patients, the low fibrinolytic capacity has been shown to be due to high levels of plasminogen activator inhibitors.[731] High PAI-1 levels are associated with a poor prognosis in young male survivors of MI,[665,732] suggesting that high PAI-1 activity may interfere with post-infarctional repair.[733] Expression of PAI-1 by hepatocytes is increased by IGF-1[734] and this mechanism may offer an explanation for the relationship of PAI-1 to proinsulin and triglycerides. This suggests a role

for the fibrinolytic system in the insulin resistance syndrome.[369,735]

Coagulation Activation

The strongest association of haemostatic risk markers with ischaemic heart disease is seen with the key factor of extrinsic coagulation, FVIIc.[720] Release of tissue factor, initiating FVII activation (FVIIc levels have been suggested to depend largely on FVIIa[736]) and subsequently increased coagulation activity, is an important feature of ischaemic heart disease. Endothelial dysfunction on the surface of atherosclerotic plaques may occur much more frequently than might be supposed from the occlusive episodes that occur in coronary arteries.[737] Nonetheless, tissue factor release secondary to endothelial damage appears to be associated with poor prognosis. Subsequent analyses within the Northwick Park Heart Study have shown that the association of FVIIc with ischaemic heart disease only applies for fatal events.[720,738,739] Thus, FVIIc levels might only reflect the extent to which overt endothelial damage, rather than dysfunction, occurs within the arterial system.

Accordingly, it has been shown that thrombosis associated with atherosclerosis (with accompanying unstable angina pectoris or MI) may be monitored by *in vivo* markers of coagulation activation.[740] Heinrich et al.[741] found the extent of ultrasound-assessed atherosclerosis of the carotid artery positively correlated with D-dimer and FDP levels. Kruskal et al.[742] found increased FDPs in patients with unstable angina and MI compared with healthy controls and patients with stable angina pectoris. High fibrinopeptide-a and F_{1+2} levels are seen in acute coronary thrombosis,[743,744] and reflect the effect of anticoagulant[745] and fibrinolytic[733] therapy of MI and the extent of coronary atherosclerosis.[746] These observations support the view that coagulation activation frequently occurs in coronary artery disease and that serial determinations of coagulation activation markers may be used to monitor the clinical course of arterial thombotic disease. However, whereas in venous thrombotic disease, an increase of coagulation activity may only be harmful to predisposed women, rupture of atherosclerotic plaques and release of large amounts of tissue factor in the circulation[737] may induce arterial occlusion even in the absence of any detectable defect of the haemostatic regulation.

4.9.4 Effects of Combined Oral Contraceptives

It is clear from this critical evaluation of the association of haemostatic factors and risk of vascular disease that it is not yet possible to ascribe absolute levels of risk to a given level of a specified risk marker. Nevertheless, interpretation of the haemostatic effects of oral contraceptives has frequently been based on changes from baseline rather than absolute measurements. For any risk marker, increased levels have been interpreted as evidence of increased risk. Moreover, decreases have frequently been misinterpreted as evidence of reduced risk. Unfortunately, the many limitations of this approach are rarely discussed. All studies with full papers in English that give data on changes of haemostatic parameters from baseline for specific oral contraceptive preparations have recently been reviewed.[747-749] Recent findings regarding the effects of low estrogen dose oral contraceptives containing desogestrel or gestodene compared with equivalent dose formulations containing levonorgestrel are mentioned in this section, as appropriate, but are reviewed in detail in section 5.7. For the purpose of the present section, a summary is given for each risk marker.

Procoagulants

Within days of use of combined oral contraceptives plasma concentrations of fibrinogen, factors V, VII, VIII, IX, X and XIII as well as von Willebrand factor increase significantly. These changes are not seen in women taking progestogen-only contraceptives.[623] The magnitude of change may approach 30% in users of high estrogen preparations. Maximum mean values that have been reported for fibrinogen and FVIII in combined oral contraceptive users are within the range for non-users. With modern low dose preparations, maximum mean changes were frequently below 10%. Modifying effects of progestogens in combined oral contraceptives appear to be limited to FVIIc, which

increase only slightly or not at all in users of low dose preparations containing levonorgestrel or norethisterone, but up to 25% in users of those containing desogestrel or gestodene. Considerable inter-individual variation in effect has been repeatedly noted in these studies. Such variation in FVIIc may underlie recent reports of variation in APC resistance according to oral contraceptive progestogen content (see section 5.7). A 25% decrease in tissue factor pathway inhibitor (TFPI) antigen and a 29% reduction in activity has recently been reported in a comparison of 40 oral contraceptive users with non-users.[750] FVII activity and F_{1+2} were also significantly increased. Either an oral contraceptive-induced reduction in TFPI is enabling increased activation of the extrinsic pathway, or activation of the extrinsic coagulation pathway is acting as a drain on TFPI levels. A greater effect of oral contraceptives on FVII levels has recently been reported in women carrying the Q allele of the R/Q353 of the *FVII* gene in a randomised comparison of 95 women taking formulations containing either desogestrel or gestodene in combination with ethinylestradiol 0.02mg.[751] The most rapid increase was seen in women using the desogestrel formulation who carried the Q allele and the slowest increase in gestodene formulation users of RR genotype. No such joint effects were apparent with the -455G/A polymorphism of the fibrinogen gene.

Anticoagulants

AT III and protein S levels decrease in users of combined oral contraceptives, but not in users of progestogen-only contraceptives. The range of values among users is shifted towards the lower range of normal by about 5 to 20%. There is evidence of a dose-response relationship with the estrogen component. In some users values below 60% of normal were found, which is in the range of congenital deficiency syndromes. It has been argued that users with low and very low inhibitor activities may have started with low baseline activities before treatment and thus be essentially inhibitor deficient. However, in a cohort analysis among women with coagulation inhibitor deficiencies it was shown that the oral contraceptive-associated effects on these congenitally low inhibitor activities are low or absent.[657]

Protein C levels increase by up to 30% in users of combined oral contraceptives. The greatest effects have been seen with the highest estrogen dose formulations, although no differences between formulations containing ethinylestradiol 0.03 and 0.02mg have been reported.

As described previously, the association between increased levels of female sex steroids and acquired APC resistance is now well established. For example, cessation of oral contraceptive use in one woman found to be APC resistant (APC sensitivity ratio 1.89) resulted in recovery of a normal APC sensitivity ratio (2.40).[685] Moreover, variation in the clinical consequences of APC resistance in an oral contraceptive user was apparent in a case report in which APC resistance, independent of factor V Leiden mutation, was found in a woman experiencing ischaemic colitis; both APC resistance and ischaemic symptoms improved with cessation of oral contraceptive use.[752] As mentioned previously, such findings draw attention to the possible influence of steroid-induced changes in other factors of the haemostatic system on the APC sensitivity ratio. Two studies found an inverse relationship between APC-sr and FVIII-clotting activity in oral contraceptive users.[690,753] However, in one of these, the Bavarian Thromboembolic Risk (BATER) Study cohort of 821 women,[690] although APC-sr correlated negatively with FVIII, the lower APC-sr in oral contraceptive users could not be attributed to increased FVIII levels since in this study oral contraceptive use did not increase FVIII levels. Oral contraceptive-induced reductions in protein S were considered unlikely to be a factor in oral contraceptive-induced APC resistance since there appeared to be little relationship between protein S levels and the APC ratio,[671,672] and there was normal APC sensitivity reported in patients with protein S deficiency.[670,671] A number of studies have examined variation in APC resistance with oral contraceptive composition, particularly with respect to progestogen con-

tent.[690,691,697,754-758] These studies are considered in detail in section 5.7.1.

In summary, although combined oral contraceptives decrease antithrombin III and protein S levels and increase protein C levels, their most prominent effect may be on the sensitivity of thrombin or fibrin generation to inhibition by APC. This may be a consequence of oral contraceptive-induced changes in factors of the extrinsic or intrinsic pathways. Variation in the sensitivity to APC appears to be influenced by differences between different oral contraceptive formulations when assessed via the extrinsic pathway but not via the intrinsic pathway. This suggests that oral contraceptive formulations differ in their effects on APC sensitivity evaluated via the extrinsic pathway as a result of their differing effects on some component of the extrinsic pathway. Possibilities include tissue factor, tissue factor inhibitor or FVII. More generally, inhibitor deficiency syndromes appear to be important in oral contraceptive associated venous thrombotic disease. Inhibitor deficiency syndromes exemplify a pre-existing latent prethrombotic state, whereas coagulation activation associated with oral contraceptive use exemplifies a non-inflammatory trigger.

Fibrinolysis

Plasminogen levels increase in users of combined oral contraceptives, depending on the dose of ethinylestradiol. Such a change has been interpreted as antithrombotic and beneficial, but evidence for this is weak. Plasma levels of t-PA and its inhibitor PAI-1 are markedly reduced in oral contraceptive users. The most consistent finding, and by far the most marked effect in oral contraceptive users, is a fall in PAI-1 levels of about 40%. This fall has been observed in women using combined oral contraceptives ranging in dose of ethinylestradiol from 0.02 to 0.05mg. Since inhibition of fibrinolysis is a dominant factor in haemostasis, combined oral contraceptives therefore appear to increase the net fibrinolytic capacity.[759] Apart from the few studies which measured the fibrinolytic response to venous occlusion,[759] evidence for a positive net effect on fibrinolysis has been taken from assessments of reaction products of fibrinolytic activity such as PAP and FDPs which increase by 40% and up to 80%, respectively.

The 40% increase in PAP levels is seen with the use of 0.02mg ethinylestradiol-containing combined preparations, as well as at higher doses. This is in spite of a clearly apparent ethinylestradiol dose-dependent increase in FDPs. A possible explanation is that the 40% stimulation of baseline fibrinolytic activity by exogenous estrogens plateaus at a rather low estrogenic potency (within the range of oral postmenopausal estrogen replacement therapy), whereas activation of coagulation appears to increase continuously with estrogen potency (within the range of oral contraceptive therapy).[760] Increased FDPs are evidence that excess fibrin generation in oral contraceptive users is of little significance in healthy women because of the increased effectiveness of the fibrinolytic system (fig. 24).

Haemostatic Activities

The most consistent finding in users of combined oral contraceptives is an increase of baseline coagulation activity as indicated by an increase of

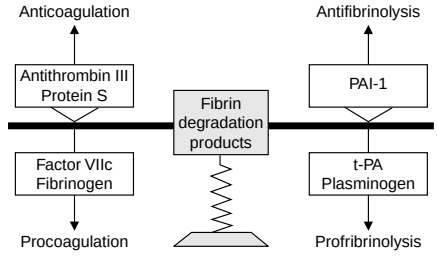

Fig. 24. The balance of haemostasis: antithrombin III, protein S and plasminogen activator inhibitor (PAI)-1 raise their sides of the balance, whereas factor VII, fibrinogen, tissue plasminogen activator (t-PA) and plasminogen lower their sides. Fibrin degradation products indicate the overall level at which the balance is set. Oral contraceptive use does not derange the balance of coagulation and fibrinolysis: in both systems a decrease of inhibiting and an increase of activating factors has been found. This suggests a shift to a new equilibrium at a higher turnover rate, as demonstrated by reaction markers such as fibrin degradation products.[761]

reaction products such as F_{1+2} by 20 to 60%. Levels of reaction markers increase with estrogen dose in postmenopausal estrogen replacement therapy (0.625 and 1.25mg conjugated equine estrogens)[762] and oral contraceptive therapy (0.02 to 0.05mg ethinylestradiol-containing preparations). It is important to note that in estrogen replacement therapy as well as in low dose oral contraceptive therapy there is no evidence of tissue factor- or IL-6–mediated haemostatic activation. FVII activation does not seem to be markedly increased in users of low dose oral contraceptives. Acute phase reactants such as fibrinogen, FVIII and PAI-1 are not significantly increased in low dose oral contraceptive users (PAI-1 is, in fact, reduced) but may be slightly reduced in estrogen replacement therapy. Thus, neither the acute phase reaction, mostly mediated by IL-6 and other cytokines, nor a marked tissue factor expression as frequently associated with endothelial damage are likely explanations for the increased baseline activity of coagulation in oral contraceptive users. Therefore, the weight of evidence points to endothelial dysfunction yielding procoagulant changes of the vascular lumenal membranes in oral contraceptive users. The exact mechanism by which this activation is mediated is unknown. However, it may be assumed that this activity can exceed the regulatory capacity of the local anticoagulant and fibrinolytic mechanisms in predisposed oral contraceptive users.[761] Nevertheless, there may be some influence of circulating factors. Inauen and colleagues[763] examined deposition of fibrin and platelets onto rabbit aorta subendothelium from flowing human blood taken from women who had been using an oral contraceptive containing ethinylestradiol 0.02mg and from women using a formulation with ethinylestradiol 0.05mg (both combined with desogestrel). Although platelet deposition did not differ between the two estrogen doses, there was significantly greater fibrin deposition at the higher estrogen dose.

Haemostatic Factors and Thrombosis in Oral Contraceptive Users

There is some evidence now regarding the clinical significance of oral contraceptive-induced changes in haemostatic factors. In the course of the population-based case-control study, the Leiden Thrombophilia Study, Bloemenkamp et al.[703] examined the possibility that women who had experienced a thrombosis while taking an oral contraceptive were hyper-responsive to oral contraceptives with regard to adverse effects on haemostatic factors. 99 premenopausal women who had been using oral contraceptives at the time of their first thrombotic episode were identified. The median time between the episode and blood collection was 18 months. At the time of blood collection, 30 of the 99 women were still using oral contraceptives. Haemostatic factor levels were compared with those in a control group of 54 oral contraceptive users and 99 non-users who had not experienced a thrombosis. Hyper-responsiveness was evaluated in several ways. For example, the proportions of those with outlying values for various haemostatic measures were compared between those using oral contraceptives at the time of blood sampling who had and had not experienced a previous thrombosis. Significant differences were found for normalised APC sensitivity ratio (aPTT method) and for protein C, there being about double the proportion of women with an APC sensitivity in the bottom 25th percentile or with a protein C activity in the top 75th percentile in those with a history of thrombosis. This shows that those who developed a thrombosis while taking oral contraceptives had a greater tendency to be APC resistant, but also tended to have elevated protein C activity. Non-significant differentials were also seen in the proportions with elevated factors VII, VIII and X and fibrinogen and reduced antithrombin III and protein S activities, there being higher proportions of those with these features among oral contraceptive users with a history of thrombosis. An alternative approach was to examine the differentials in haemostatic measures between the 4 groups studied. Only the APC sensitivity ratio differed significantly between oral contraceptive users with and

without a history of thrombosis (reduced in the latter group), although this differential was not as great as that seen between non-users with and without a history of thrombosis. A greater differential (although not significant) was seen for FVII, AT III and protein C in oral contraceptive users compared with non-users, with and without a history of thrombosis (higher levels of FVII and protein C and lower levels of AT III were seen in oral contraceptive users and in those with a previous thrombosis). It was also noted that the median number of variables for which a woman was a high responder was highest among current oral contraceptive users who had experienced a previous thrombosis. Overall, these findings suggest that oral contraceptive users who had experienced a thrombosis were more likely to be APC resistant (aPTT - intrinsic pathway evaluation). Though not significant, disproportionate elevations in factors VII, VIII and X and fibrinogen and reductions in antithrombin III and protein S activities were also indicated in these women. Possibly oral contraceptive users who experience a thrombotic event have a generalised hyper-responsiveness to oral contraceptive estrogens.

Risks of DVT associated with oral contraceptive use and high FVIII levels were explored, again in the context of the Leiden Thrombophilia Study, in 155 women aged 15 to 49 years who had experienced a DVT, and in an age-matched control group of 169 women.[764] 70% of cases had been using oral contraceptives within one month of their thrombosis, compared with 38% of controls. 36% of cases had high FVIII levels compared with 17% of controls. The odds ratio of VTE for oral contraceptive use without raised FVIII was 4.0, and for raised FVIII in the absence of oral contraceptive use 5.3. The joint effect of oral contraceptive use and high FVIII level gave an odds ratio of 10.3 (CI 3.7 to 28.9). Blood sampling in this study was 15 months after the event, so some of those using oral contraceptives at the time of their event would have been using oral contraceptives at the time of venepuncture (about 30), whereas others would not. Therefore, the study may relate more to the importance of underlying variation in FVIII levels rather than oral contraceptive-induced changes.

The Role of Haemostasis

It is noteworthy that several established mechanisms of haemostasis-mediated vascular risk are not affected by oral contraceptive use. Some putative mechanisms may even be affected in a beneficial direction. FVIII and, to a somewhat lesser degree, fibrinogen levels are only substantially increased with high estrogen-dose oral contraceptives but are essentially unchanged with low dose preparations. PAI-1 levels and activity were found to be markedly reduced in users of oral contraceptives, suggesting that an acute phase reaction is not a likely pathogenetic mechanism of oral contraceptive-induced thromboembolism. Interestingly, this mechanism is associated with a rather rapidly progressing, extensive clotting such as in septic shock or rupture of an atherosclerotic plaque and oral contraceptive-associated thromboembolic disease may progress at a much slower speed both in the venous and arterial system.

The most likely explanation for the tonic low grade activation of coagulation in oral contraceptive users is an effect on endothelial function yielding activation of the lumenal membranes and expression of membrane bound cofactors. This will result in a local shift of both coagulation and fibrinolysis to a higher level of activity which may only induce venous thrombosis in the presence of an insufficient capacity of antithrombotic, i.e. anticoagulant or fibrinolytic mechanisms.

5. The Third versus Second Generation Pill Controversy

The WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception was a hospital-based, case-control study of risks of nonfatal VTE, MI, ischaemic stroke and haemorrhagic stroke, undertaken between 1989 and 1993.[16] Twenty-one centres throughout the world recruited a total of 3276 cases and 8811 controls making this the largest study of its kind ever undertaken. Among the many findings generated by this study was the unexpected observation that, com-

pared with users of oral contraceptives containing mainly levonorgestrel as progestogen, users of formulations containing the more recently introduced progestogens, desogestrel or gestodene, were overrepresented among cases of VTE. Odds ratios for VTE relative to users of low estrogen dose levonorgestrel formulations were 2.2 for users of desogestrel formulations and 3.0 for users of gestodene formulations.[85] This was surprising because it had been accepted, hitherto, that the oral contraceptive estrogen component was entirely responsible for risk of VTE, and the comparison involved formulations of very similar estrogen dose. This finding was first made public by the UK Committee on Safety of Medicines on the 18th October 1995. In response to this, there has followed one of the most intense periods of debate regarding the cardiovascular effects of combined oral contraceptives in the history of oral contraceptive prescribing. Work on the present review began in November 1995 and was intended to provide an indepth background to the arguments that were even then developing. At the time of finalising this work (August 2000) a number of avenues still remain to be explored. It is to be hoped, however, that the debate and the rapid acquisition of new information will continue and will eventually culminate in an agreed understanding as to exactly why the combined oral contraceptive causes venous thrombosis and whether the progestogen component has a role in this.

This controversy over the relative safety of different low-estrogen dose oral contraceptives has encompassed almost every aspect of research into the cardiovascular effects of oral contraceptives, from epidemiology to basic science. Rather than refer to aspects of the controversy according to the section structure of this review, we have chosen to present a single formal overview that attempts to provide an accurate account of the extensive published evidence, arguments and issues raised by the original WHO Collaborative Study observation. The intention is to concentrate on the scientific evidence, rather than issues relating to regulatory agency recommendations, prescribing practice or estimates of absolute risk, which have been dealt with by others.[195,765-768]

The emphasis in this section is on previously published data and opinion. At every stage in the developing debate, summary reviews have appeared, some of a general nature, others promoting a specific interpretation of the data.[29,87,769-781] The present overview adds to this body of work by considering, in addition to earlier work, developments since 1998. These have included the publication of several new epidemiological studies and reanalyses of data from earlier studies. The most significant development, however, has been in the evaluation of differences in effects of the formulations in question on haemostatic function, which have been put forward to provide a plausible biological mechanism that could underlie the observed differences in risk.

In what follows we have attempted to provide an accurate account of the published material. However, we inevitably have our own opinions regarding some of these issues. To distinguish these from the basic summary, these are interpolated as 'Authors' notes'. The concluding summary also represents our opinions. One basic premise we have adopted is that use of the terms 'third generation progestogen' (e.g. desogestrel or gestodene), 'second generation progestogen' (e.g. levonorgestrel), or 'first generation progestogen' (e.g. norethisterone) is imprecise when attempting to distinguish the effects of combined formulations that contain them. For example, among low estrogen dose (0.03 to 0.04mg) formulations, very low dose (0.5mg) norethisterone-, norgestimate- or cyproterone-containing formulations affect lipid metabolism very similarly to formulations containing so-called third generation progestogens, but have generally been considered in isolation or alongside formulations containing second generation progestogens. Equally, very low estrogen dose (0.02mg) formulations containing desogestrel or gestodene may have less impact on haemostatic variables than their low estrogen dose equivalents, but have been considered as 'third generation' formulations. Therefore, there may be potentially important dif-

ferences between formulations independent of their third or second generation status. If, at some future date, a specific property of oral contraceptive progestogens is identified that specifically confers significant risk of VTE, and this property parallels the third versus second generation distinction, then this terminology may then be justified. At present, however, there is no such distinguishing property. Moreover, as should be apparent from the examples given here, the terms 'second generation' and 'third generation' obscure distinctions that may be informative with regard to cardiovascular disease risk in oral contraceptive users, and they have, in any case, been inconsistently applied to different groups of oral contraceptives in different studies.[32,52] Instead of these general terms, formulations will be referred to in this section according to composition.

5.1 The World Health Organization (WHO) Collaborative Study

The core observation that initiated the current phase of debate and investigation regarding the relative safety of different low estrogen dose oral contraceptives was the finding from the WHO Collaborative Study that formulations containing desogestrel and gestodene were associated with an odds ratio for risk of VTE that was at least twice that for those containing levonorgestrel.[32] Specifically: non-users comprised 397 cases and 1519 controls; users of desogestrel formulations 35 cases and 28 controls (including 8 cases and 1 control using a combination of ethinylestradiol 0.02mg with desogestrel 0.15mg); users of gestodene formulations 36 cases and 28 controls; users of levonorgestrel formulations 137 cases and 203 controls (including 1 case and 2 controls using a norgestimate-containing formulation); and users of other formulations 164 cases and 201 controls.[85] The other formulations included predominantly combined oral contraceptives containing estrane progestogens in combination with ethinylestradiol 0.05mg. Analyses were undertaken of data for: (i) all centres; (ii) centres outside the Oxford, UK region (for which only hospital-based controls were identified); (iii) centres within the Oxford region with hospital-based controls, and (iv) centres within the Oxford region with community-based controls. In these datasets, odds rations (adjusted for BMI and, variously, varicose veins, hypertension in pregnancy or cigarette smoking) for VTE in desogestrel and gestodene formulation users combined, compared with levonorgestrel combination users were: (i) 2.7 (95%CI 1.6 to 4.6), (ii) 5.2 (2.0 to 13.7), (iii) 2.2 (1.1 to 4.2) and (iv) 1.4 (0.6 to 3.1). In the Oxford region, incidence rates per 100 000 women-years of nonfatal VTE were estimated to be 4 for women not taking oral contraceptives, 10 for women taking levonorgestrel formulations and 21 for women taking desogestrel or gestodene formulations.

5.2 Immediate Questions Generated by the WHO Collaborative Study Findings

Controversially, these observations were publicised prior to peer-reviewed publication, so even before the data could be assessed questions and concerns had been raised in relation to the influences of bias or confounding. By the time the results were published, these concerns were already beginning to develop their own lines of controversy.[782] These questions, and questions posed in the WHO Collaborative Study publication itself, set the agenda for the intense and often heated phase of debate and investigation that has followed. These questions are summarised as follows:

- given that an investigation into risks associated with different formulations was only a secondary objective of the WHO Collaborative Study, and given that odds ratios of only around 2 were being considered, could the findings be reproduced independently?[85]
- could the findings have been influenced by unrecognised confounding or bias?[85,783]
- could the findings be explained by preferential prescribing of newer formulations containing desogestrel or gestodene (which were perceived by prescribers to be safer) to women at increased risk of VTE?[783]

- could the findings be explained by preferential prescribing of desogestrel or gestodene formulations to first time users, so that women with a pre-existing, unrecognised tendency to thrombosis experienced their first event in association with use of a desogestrel or gestodene-containing formulation?[784]
- could the findings be explained by an unrepresentatively low risk among users of older formulations, as a result of a progressive loss from this group of users with a tendency to thrombosis and consequent selection of those without such tendency?[785]
- if risk of VTE resides primarily in the estrogen component of the combined oral contraceptive, why was there a higher risk in users of lower estrogen dose formulations containing desogestrel or cyproterone, compared with users of higher estrogen dose formulations containing the same progestogens at the same dose?[85]
- might there be a commensurate reduction in the risk of arterial disease in users of desogestrel or gestodene-containing formulations?[85,782,783,786,787]
- was there a biological mechanism that could account for the observed difference in risk?[85,783]

5.3 Other Studies

Further analyses comparing risks of VTE between users of different oral contraceptive formulations have come from a study requested by regulatory authorities in response to the WHO Collaborative Study findings [the General Practice Research Database (GPRD) Study], from studies not specifically initiated to address the issues concerned, but which nevertheless included information that enabled comparisons to be made between risks associated with different formulations (the Leiden Thrombophilia Study and others), from a study running concurrently with and of comparable design to the WHO Collaborative Study (the Transnational Study), and from studies newly-undertaken in response to the WHO Collaborative Study (MediPlus and PHARMO database analyses and Danish Hospital Register studies). Results from the WHO Collaborative Study and these further studies and analyses are summarised and referenced in table XII.

5.3.1 The UK General Practice Research Database Analyses

In response to the WHO Collaborative Study findings, the Medicines Control Agency commissioned an analysis of data from the GPRD. Between January 1991 and November 1994, 80 cases of nonfatal VTE were identified among 238 130 otherwise healthy women drawn from 365 general practices.[28] Findings were published concurrently with those of the WHO Collaborative Study. 75 cases and 300 controls were current oral contraceptive users. In a nested case-control analysis of these data (with matching for age, practice and index date, and adjustment for smoking and body mass index), odds ratios of 2.2 (95%CI 1.1 to 4.4) and 2.1 (1.0 to 4.4) were found for VTE among users of low estrogen dose formulations containing desogestrel or gestodene, respectively, compared with levonorgestrel formulation (this doubling of risk was also apparent in a GPRD-based study of risks of VTE among women who had used postcoital contraceptive pills at some time between January 1989 and October 1996[788]).

A further analyses utilising the GPRD database was undertaken by Farmer and colleagues.[34,793] This included records from between January. 1992 and June 1997. In a nested case control analysis, adjusted odds ratios of 1.0 (0.7-1.7) and 1.3 (0.8-2.1) obtained for VTE among users of low estrogen dose formulations containing desogestrel or gestodene, respectively, compared with formulations containing levonorgestrel. The authors ascribed the lower odds ratios obtained in this analysis compared with the earlier analysis of Jick and colleagues[28] primarily to age matching of controls to within 1-year age bands, rather than 5-year age bands. Most recently, Farmer and colleagues[798] utilised data from the GPRD database, covering the period January 1993 to December 1998, to assess the effect on rates of venous thromboembolism of the precipitous fall in use of desogestrel and gestodene-containing formulations in the UK after October 1995. Although use of these formulations

Table XII. Odds ratios or relative risks for venous thromboembolism for 'third' versus 'second-generation' oral contraceptive (OC) users. Odds ratios for low-estrogen dose desogestrel compared with levonorgestrel-containing formulations are shown where these are available. Otherwise the comparison closest to this is reported. Matched and adjusted odds ratios are given where available

Study period	Source of data	No. of cases OC users only	Odds ratio (95% CI)
1982-1995	Amsterdam anticoagulation clinics[36]	51	1.9 (0.8-4.5)
1986-1995	PHARMO Study (new users)[75]	33	4.2 (1.7-10.2)
1988-1992	Leiden Thrombophilia Study [72]	57	2.2 (0.9-5.4)
1989-1993	WHO Collaborative Study [32]	101	2.3 (1.1-4.9)
1989-1996	GPRD analysis (post-coital pill users)[788]	8	1.8 (0.3-11.2)
1990-1991	Meditel - UK[789]	30	1.3 (1.2-1.8)
1990-1998	New Zealand mortality records[790]	15	2.1 (0.5-9.8)[a]
1991-1994	GPRD analysis[28]	75	2.2 (1.1-4.4)
1991-1995	Transnational Study (1st analysis)[52]	318	1.5 (1.1-2.1)
1991-1995	Transnational Study (2nd analysis)[53]	334	1.9 (1.2-2.9)
1991-1995	Transnational Study (with full exposure history)[81]	339	1.1 (0.6-2.0)
1991-1995	Transnational Study (repeated users of current formulation)[81]	74	0.6 (0.3-1.2)
1991-1995	Transnational Study (switchers from '3rd or 2nd generation')[81]	81	1.3 (0.7-2.4)
1991-1995	MediPlus - UK[86]	83	1.2 (0.7-2.2)
1992-1997	MediPlus - UK[791,792]	99	1.1 (0.5-2.6)
1992-1997	GPRD analysis[34,793]	241	1.0 (0.7-1.7)
1992-1995	MediPlus - Germany[794]	42	0.8 (0.4-1.6)
1994-1995	Danish Hospital Records[31]	172	1.4 (0.8-2.5)
Other information			
1977-1998	North Jutland and Viborg, Denmark[795]	68	2.0 (0.7-5.8)[b]
1992-1994	South Sweden[796]	6	3.3 (0.98-9.0)[c]
1992-1996	Netherlands clinical trial: cerebral sinus thrombosis[797]	34	'2.0 to 1.0'[d]
1995-1998	Italian anticoagulation clinic [71]	42	'No significant effect'

a Crude odds ratio estimated from figures given. Relative to non-users (9 cases, 86 controls), the adjusted odds ratio for fatal pulmonary embolism for desogestrel/gestodene formulation users was 2.9 times higher than for levonorgestrel formulation users.

b Crude odds ratio – controls taken from a non-concurrently recruited group of blood donors.

c Observed to expected ratio based on regional prescribing information.

d Observed to expected ratio based on national prescribing information.

CI = confidence interval; **GPRD** = General Practice Research Database; **WHO** = World Health Organization.

fell from 53 to 14%, there was no accompanying change in rates of VTE.

5.3.2 The Leiden Thrombophilia Study

Although not designed to examine differences in risk associated with use of different oral contraceptive formulations the Leiden Thrombophilia Study provided useful information that was published concurrently with the WHO Collaborative Study findings. 126 women aged 15 to 49 years, who had experienced a DVT were compared with 159 controls.[72] Compared with users of other low estrogen dose formulations, there was an age-adjusted odds ratio for DVT of 2.5 (95%CI 1.2 to 5.2) among users of desogestrel-containing formulations. Comparing equivalent low estrogen dose levonorgestrel and desogestrel formulation users, an adjusted odds ratio of 2.2 (0.9 to 5.4) was found for the desogestrel formulation users.

5.3.3 The Transnational Research Group on Oral Contraceptives and the Health of Young Women

Concurrently with the WHO Collaborative Study, another major investigation of cardiovascular dis-

ease in oral contraceptive users was proceeding. The Transnational Study was instigated by the German health authorities to investigate both a cluster of case reports of VTE in users of gestodene-containing oral contraceptives, and the health implications of a reported increase in the plasma half-life of the oral contraceptive estrogen, ethinylestradiol, by gestodene. To enable comparison with the WHO Collaborative Study, the Transnational group adopted an identical study design to that of the WHO Collaborative Study. The Transnational Study would have published entirely independently of the WHO Collaborative Study, albeit at a later date. However, at the request of the Medicines Control Agency, in the wake of the WHO Collaborative Study results first becoming known, a preliminary analysis of the Transnational data was undertaken.[794] There then followed the marked changes in oral contraceptive usage in late 1995, after the findings from the WHO Collaborative Study, the Transnational Study, the GPRD Study and the Leiden Thrombophilia Study were made known, and it was judged necessary to prematurely terminate the VTE component of Transnational Study. Recruitment of cases continued until October 1995 and of controls until December 1995.[53]

The first published report from the Transnational Study concerned 471 cases of fatal or non-fatal VTE and 1772 controls (matched to the cases within 5-year age bands) from 10 centres, primarily in Germany and the UK, accrued up to 3rd October 1995.[52] Controls were both hospital- and community-based. The odds ratio (adjusted for age, smoking, alcohol use, study centre, BMI and duration of oral contraceptive use) for VTE among users of low estrogen dose desogestrel- or gestodene-containing formulations compared with those taking levonorgestrel- or norethisterone-containing formulations was 1.5 (95%CI 1.1 to 2.1).

In a further analysis from the Transnational Study, restricted to the centres in Germany and the UK and including all those recruited into the study up to the time of its termination in December 1995, 505 cases were compared with 1877 controls, and users of individual oral contraceptive formulations were compared with users of levonorgestrel-containing formulations.[53] The following odds ratios (adjusted for age, BMI, smoking, alcohol use and duration of use of oral contraceptives prior to that currently used) were found: gestodene-containing formulations (58 cases) 1.68 (95%CI 1.10 to 2.58); desogestrel-containing formulations with ethinylestradiol 0.03mg (64 cases) 1.86 (1.23 to 2.88); desogestrel-containing formulations with ethinylestradiol 0.02mg (15 cases) 1.57 (0.80 to 3.07); norgestimate-containing formulations (19 cases) 1.85 (0.95 to 3.58); formulations containing ≥0.05mg ethinylestradiol (38 cases) 1.94 (1.18 to 3.21).

Three further analyses of the Transnational Study data endeavoured to provide more rigorous standardisation for possible effects related to duration and pattern of previous oral contraceptive usage than had been attempted in other studies.[74,81,799] The two concerned with duration effects[74,81] are considered in detail in section 5.4.5. In the third analysis,[799] first-time use, repeated intermittent use and switching between formulations was distinguished, with comparisons made between those who had intermittently used desogestrel/gestodene formulations and those who used levonorgestrel formulations, and between those who had switched from the latter to the former and from the former to the latter. In these 3 analyses, the difference in risk of VTE between users of desogestrel or gestodene formulations and users of other low estrogen dose formulations was no longer apparent.

5.3.4 MediPlus Analyses in the UK

A series of analyses were undertaken by Farmer and colleagues deriving data from patient records collated under the Meditel practice management system. In an earlier analysis of records from 1990 to 1991 for 693 705 women in the UK aged 14 to 45 years, absolute risks of VTE associated with oral contraceptive use were explored, but risks associated with different formulations were not reported, no such differences having been found.[15] In response to the WHO Collaborative Study findings, and confirmatory findings from other studies published concurrently, a comparison of VTE risks between users of formulations containing ethinyl-

estradiol 0.03mg and desogestrel or gestodene, and low estrogen dose formulations with levonorgestrel as progestogen was reported.[789] There were 37 cases of VTE (distinguished by prescription of anticoagulants or admission to hospital for pulmonary embolism), 30 among oral contraceptive users. The relative risk for VTE in desogestrel and gestodene formulation users compared with levonorgestrel formulation users was 1.3 (95%CI 1.2 to 1.8). In a second analysis, the MediPlus database was employed, which at the time, comprised records drawn from 143 general practices in the UK that used the Meditel system.[86] 83 cases of thrombosis occurring among 491 908 women between September 1991 and September 1995 in women exposed to combined oral contraceptives were evaluated compared with 313 controls matched to the same year of birth and for use of an oral contraceptive on the day that the thrombosis occurred in the corresponding case. In a nested case-control analysis, the odds ratio (adjusted for BMI, change in type of oral contraceptive prescribed in the preceding 3 months of the thrombotic event, number of cycles prescribed, previous pregnancy, concurrent disease or previous use of 'morning-after' contraception) for VTE in users of low estrogen dose desogestrel or gestodene-containing formulations, compared with users of mainly levonorgestrel-containing formulations was 1.18 (95%CI 0.66 to 2.17). The authors attributed the difference between their findings and those from other studies to the close age matching they employed. This supposition was questioned since it seemed unlikely that the differences in closeness of age matching between the studies were sufficient to account for complete disappearance of the effect,[775,800] and tighter age matching in both the WHO and GPRD Studies made little difference to the findings.[801,802] The study was also criticised for the lack of validation of the cases studied, although the authors argued that it was unlikely that anticoagulant therapy would be continued if there was doubt over whether a thrombosis had occurred.[803]

At the time of writing, the most recently published analysis from the MediPlus database has involved a nested case-control analysis of 99 cases of idiopathic VTE, and 366 controls, matched for practice, year of birth and exposure to oral contraceptives coincident with that of the case.[791] Records accumulated between January 1992 and March 1997 were considered. A validation exercise was also undertaken with regard to the criteria on which a diagnosis of VTE were based. The odds ratios (adjusted for BMI category, smoking status, DBP and noncontraceptive prescriptions), relative to users of low estrogen dose levonorgestrel formulations, for low estrogen dose desogestrel and gestodene, and very low dose desogestrel formulations were 1.1 (95%CI 0.5 to 2.6), 1.1 (0.5 to 2.4) and 1.1 (0.4 to 3.4), respectively.

5.3.5 MediPlus Analysis in Germany

In an analysis similar in principle to that described for the MediPlus database in the UK, Farmer and colleagues[792,794] undertook an analysis of risks of venous thrombosis in German women, with records concerning 101 797 women drawn from 451 practices. 42 cases of thrombosis (distinguished by subsequent anticoagulant treatment) occurring between October 1992 and September 1995 in women exposed to combined oral contraceptives were evaluated in comparison to 168 controls matched to the same year of birth and for use of an oral contraceptive on the day that the thrombosis occurred in the corresponding case. The odds ration (unadjusted because of incomplete information in a substantial proportion of cases) for VTE in users of desogestrel or gestodene-containing formulations with ethinylestradiol 0.03mg, compared with users of levonorgestrel or norethisterone-containing formulations was 0.77 (95%CI 0.38 to 1.57).

5.3.6 Case Control Analysis of Data from the Danish Hospital Register

With follow-up of cases identified using the Danish Hospital Register system, Lidegaard and colleagues[31] identified 375 women aged 15 to 44 years who had experienced a VTE, with no known predisposing factors, in the period 1994 to 1995. 1041 controls were available for comparison. The odds ratio (adjusted for duration of oral contraceptive use) for VTE in users of desogestrel/gestodene

formulations was 2.3 (95%CI 1.6 to 3.2), and for users of levonorgestrel or norgestimate formulations 1.6 (1.0 to 2.5). The odds ratio for the latter compared with the former group was 1.4 (0.8 to 2.5).

5.3.7 The Dutch PHARMO System

This database system incorporates information on drug dispensing and hospital admissions for 450 000 residents of 8 Dutch cities and was used to identify women aged 15 to 49 years who had ever used oral contraceptives between 1986 and 1995 with no history of VTE and other relevant exclusion criteria.[75] All first episodes of exclusive use of low estrogen dose desogestrel or gestodene and levonorgestrel-containing formulations were identified as well as first occurrences of VTE. 27 cases of VTE were identified in 29 986 person years of observation among desogestrel or gestodene formulation users, in contrast to 6 cases in 24 953 person years among levonorgestrel formulation users, giving a relative risk (adjusted for year and age) of 4.2 (95%CI 1.7 to 10.2).

5.3.8 Dutch Anticoagulation Clinics

In an evaluation of 150 cases and 511 controls drawn from women referred for suspected VTE to two specialist centres in Amsterdam, the Netherlands, between September 1982 and October 1995, the odds ratio (adjusted for age, family history, centre and calender time) for VTE in users of desogestrel- or gestodene-containing formulations compared with users of levonorgestrel formulations was 1.9 (0.8 to 4.5).[36]

5.3.9. New Zealand Mortality Records

In response to concerns on the part of the New Zealand Ministry of Health about increased case reporting of venous thromboembolism in users of desogestrel- or gestodene-containing oral contraceptives, an analysis of national mortality records concerning death from pulmonary embolism was undertaken [790]. Deaths recorded between January 1990 and August 1998, yielded 36 eligible cases of whom 3 had been taking levonorgestrel and 12 desogestrel- or gestodene-containing formulations within 3 months of death. Controls were drawn from the family physician group practice records of each case. Compared with controls, adjusted odds ratios of 5.1 (1.2-21.4) and 14.9 (3.5-64.3) were obtained for users of levonorgestrel or desogestrel/gestodene containing oral contraceptives, respectively.

5.3.10 Other Studies

An over-representation of desogestrel or gestodene oral contraceptive users was also noted in a study in the Netherlands of 34 cases of cerebral sinus thrombosis using oral contraceptives.[797] The observed proportion of desogestrel or gestodene formulation users among the cases was 56%, whereas the proportion expected from prescription information for the Netherlands during the period in question was 38%, an observed to expected ratio cited as '2 to 1'. In a study of interactions between prothrombin and factor V gene mutations and oral contraceptive use, undertaken at a thrombosis centre in Italy, 112 patients, aged 15 to 48 years and referred between April 1995 and April 1998, were compared with 179 controls.[71] The overall odds ratio for VTE and oral contraceptive use, in the absence of known genetic risk factors for thrombosis, was 4.6 (95%CI 2.6 to 8.0). 73% of patients and 80% of controls used formulations containing desogestrel or gestodene. Although no figures were presented, the authors stated that there was no association between venous thrombosis and the type of oral contraceptive used.

Two studies have been based on recall of patients identified from hospital discharge records with subsequent blood sampling for the evaluation of hereditary thrombophilia. In the counties of North Jutland and Viborg, Denmark, 67 eligible cases, accumulated since 1977, were identified and compared with 134 blood donor controls.[795] Compared with non-users, the odds ratio for VTE (adjusted for smoking, BMI and parity) was 5.2 among users of oral contraceptives other than desogestrel or gestodene, and 48.6 among users of desogestrel or gestodene formulations. This study was criticised for the absence of coordinated timing between case and control oral contraceptive use, uncertainties over potential relationships between

blood donor status and oral contraceptive use, and its dependence on long term recall of oral contraceptive use.[775] In a study based on referrals to a single hospital in south Sweden, 27 eligible cases of VTE were considered, with reference data provided by statistical information for the population served by the hospital in question.[796] The authors noted that 5 of the 6 cases taking oral contraceptives took desogestrel formulations and estimated an odds ratio of 3.3 (95%CI 0.98 to 9.04) for women using desogestrel oral contraceptives, and 0.8 (0.26 to 2.00) for use of any contraceptives.

The use that has been made of national statistics for VTE should also be mentioned. Analyses of mortality in The Netherlands and the UK showed a steep increase in incidence among young women, which was centred around 1990.[804,805] This increase was also apparent in hospital admission rates for VTE in Denmark.[806] It is noteworthy that it was also apparent in relation to the incidence of VTE in pregnancy in Denmark,[84] which would have been unaffected by oral contraceptive use. In the study of VTE in pregnancy, the increase was ascribed to changes in diagnostic testing. This was also the conclusion drawn from an analysis of hospital admission and mortality rates between 1975 and 1998 for venous thromboembolism in the UK Oxford region.[807] The authors concluded that there was no evidence for changes associated with oral contraceptive use. Summarising the problems inherent in such studies, Hannaford concluded, '[t]hese studies provide little useful information about whether there is likely to be a causal relationship between use of steroid contraception and cardiovascular disease'.[19]

5.4 Possible Roles of Confounding and Bias

To the extent that differences in risk of VTE between users of low estrogen dose desogestrel- or gestodene-containing formulations and levonorgestrel formulations apparent in the majority of studies described above could be explained by biases or confounding factors, the possibility that these differences reflect causation on the part of desogestrel or gestodene formulations would be commensurately less likely.

5.4.1 Prescribing Bias

If prescribing bias was operating, it would be expected to have led to preferential prescribing of desogestrel- or gestodene-containing formulations to women perceived to be at greater risk of VTE. Evaluations of such prescribing bias have indicated that desogestrel- or gestodene-containing formulations had been prescribed in preference to levonorgestrel-containing formulations for women starting oral contraception, older women, those with a personal or family history of venous thrombosis, clinical venous signs, inflammatory disease, anaesthesia or plaster cast, obesity, diabetes mellitus, standing working position, alcohol abuse or smoking.[808-813] It was noted that no one study was able to adjust for the effects of every one of these factors.[769] In the WHO Collaborative Study analysis, odds ratios were adjusted for BMI and, variously, varicose veins, hypertension in pregnancy or cigarette smoking with little effect on the odds ratios.[85] In the Transnational Study, adjustment was made for age, BMI and cigarette smoking, again with little effect on the odds ratios.[52] In the GPRD analysis, cases and controls were matched for age, general practice and index date, and adjustment was made for smoking and BMI with little effect on the risk estimates.[28] In the Leiden Thrombophilia Study, adjustment for age, factor V Leiden mutation, family history of VTE and previous pregnancy had little effect on the higher odds ratio in desogestrel formulation users compared with users of other formulations.[72]

The issue of prescribing bias becomes particularly important when considering differences in risk between women using oral contraceptives for the first time, who have used the oral contraceptive for a short duration and who have not had a previous pregnancy. In these women, duration of use of oral contraceptives, previous oral contraceptive exposure and previous exposure to high estrogen levels will not be responsible for differences in risks between users of different oral contraceptive

formulations. Risks of VTE in such women are considered in section 5.4.5.

Authors note: the demonstration by Petitti et al.[14] that the greater the prevalence of a risk modifier in the group under consideration, the less are standard techniques of adjustment able to eliminate the influence of that modifier on risk estimates, should be borne in mind in relation to these issues. The only way of ensuring comparability between groups is to stratify for as many potential confounding factors as possible. It is appreciated that this may prohibitively difficult. However, in the absence of such an exercise, the only certainty at present is that desogestrel- and gestodene-containing formulations have been the subject of preferential prescribing.

5.4.2 Diagnostic Bias

Diagnostic bias was considered in the WHO Collaborative Study with separate analyses for pulmonary embolism and DVT. Among levonorgestrel formulation users, odds ratios were 3.8 and 2.7, respectively, and among users of desogestrel and gestodene formulations, they were 11.6 and 5.5, respectively.[85] In the first report from the Transnational Study, odds ratios of 2.0 (95%CI 1.2 to 3.4) and 1.2 (0.8 to 1.9), respectively, for pulmonary embolism and DVT in users of desogestrel- or gestodene-containing formulations compared with those taking mainly levonorgestrel-containing formulations were found.[52] In these two studies, the higher odds ratio for pulmonary embolism, the condition more likely to be accurately diagnosed, argues against the operation of diagnostic bias. In the GPRD analyses, separate analyses were undertaken for definite and possible VTE. Odds ratios of 2.2 (1.0 to 4.7) and 2.2 (0.7 to 7.3), respectively, were obtained.[28]

5.4.3 Referral Bias

In general, referral bias in hospital-based case-control studies would be expected to result from a greater tendency to refer women taking oral contraceptives to hospital for investigation. For referral bias to account for the observed differences between users of desogestrel or gestodene formulations and users of levonorgestrel formulations, it would then have to be argued that women taking the former would be more likely to be referred, possibly on account of being first-time users or having more risk factors.[769] Such considerations would also encompass diagnostic bias. Referral and diagnostic bias were minimised in the study of Bloemenkamp et al.[36] in which only women referred to specialist centres for suspected VTE were considered. As described in section 2.1.3, among 150 cases and 511 controls, the odds ratio (adjusted for age, family history, centre and calender time) for VTE in users of desogestrel- or gestodene-containing formulations compared with users of levonorgestrel formulations was 1.9 (0.8 to 4.5).

5.4.4 Biases in Control Group Selection

The different odds ratios encountered in the WHO Study, according to whether hospital or community controls were analysed, were explored further in the first report from the Transnational Study.[52] Odds ratios for VTE in users of desogestrel- or gestodene-containing formulations compared with those taking levonorgestrel- or norethisterone-containing formulations were 1.4 (95%CI 1.0 to 2.0) with community controls and 1.7 (1.2 to 2.5) with hospital controls, suggesting little influence of control group selection in this study.

5.4.5 Potential Biases Relating to Previous Oral Contraceptive Exposure

Issues relating to possible biases associated with variation in previous oral contraceptive exposure have been prominent in the debate over differentials in risks of VTE between users of different low estrogen dose oral contraceptive formulations. With regard to duration of oral contraceptive use, conventional methods have tended to treat this as a continuous linear variable, the underlying assumption generally being that increasing duration of use might increase the likelihood of disease developing. As mentioned in section 2, it was recognised early in the debate that manifestations of the influence of duration of use on VTE risk could be more complex.

Two complementary hypotheses may be distinguished. The first relates to the increased likelihood of a thrombotic event manifesting in the early

months or years of first use of oral contraceptives. It is proposed that many thrombotic events in oral contraceptive users are linked to a hitherto undetected tendency to thrombosis. The chances of this tendency being manifested are greatly increased on exposure of the woman to the prothrombotic effects of estrogens – either as a consequence of oral contraceptive use or pregnancy. Therefore, if a woman is going to experience a thrombotic event while taking oral contraceptives, this will most likely occur relatively soon after commencement of use of an oral contraceptive for the first time by a woman who has not been pregnant before. The possibility for bias then results from the possibility that first-time users of oral contraceptives are more likely to be prescribed more recently-introduced formulations which have been promoted on the basis of, for example, lower dosage, greater safety or fewer adverse effects. These formulations then become associated with a greater incidence of thrombosis because they are used by a greater proportion of women with an unrecognised tendency to thrombosis.

The second hypothesis stems from the consequences of this increased risk on first exposure to estrogens. It may be expected that if a woman has had a thrombosis, either during pregnancy or oral contraceptive use, she is less likely to use combined oral contraceptives in the future. Those with a susceptibility to thrombosis, revealed by pregnancy or oral contraceptive use, will therefore not be among those who continue using oral contraceptives in the longer term. With increasing time since introduction of a particular formulation, a population of long term users of that formulation will accumulate who are less and less likely to develop a thrombosis, all those with such a tendency having had a thrombosis and consequently dropped out of the population of users. This so-called attrition of susceptibles results in a population of oral contraceptive users who have remained healthy.

These two complementary hypotheses lead to the prediction that, in any given study, short term, first-time ever users of combined oral contraceptive will have a risk of thrombosis higher than that for the group as a whole and longer term users will have a lower risk. Other predictions may be made. After adjustments for the effects of age, it may be expected that younger users will be at greater risk than older users, since the younger users will include a greater proportion of susceptible short term, first-time users, whereas older users will include a greater proportion of selected healthy users. Equally, first-time users of a particular formulation may be expected to include a greater proportion of short term users than women who have changed formulations in the past, so similar risk differentials may be seen between first-time and other users. However, it should be borne in mind that first-time users could include a proportion of long term users who have never felt it necessary to change their formulation. There may also be a differential between women who have not been pregnant before and those who have. It then remains to determine whether or not the differential in risk of VTE between users of desogestrel- or gestodene-containing formulations compared with levonorgestrel-containing formulations remains after these factors have been taken into account.

First-Time Ever Use, Age, Previous Pregnancy and Duration of Use Together

Classification by first-time ever use, age, previous pregnancy and duration of use was undertaken in the analysis of data gathered under the PHARMO system, which was restricted to first-time ever users.[75] The odds ratio for VTE among first-time ever users of low estrogen dose desogestrel- or gestodene-containing formulations compared with users of equivalent levonorgestrel-containing formulations was 4.2 (95%CI 1.7 to 10.2). Among women aged <25 year or ≥25 years, odds ratios were 8.5 (1.1 to 65.5) and 2.8 (0.8 to 10.8), respectively. Among women without and with a previous pregnancy, odds ratios were 5.2 (1.5 to 13.8) and 2.9 (0.8 to 10.8), respectively. Among those who had been using the oral contraceptive for <1 year and for ≥1 year, the odds ratios were 3.3 (1.2 to 8.9) and 8.1 (1.0 to 63.6), respectively (although the higher odds ratio in the longer term users was contrary to expectation and was not confirmed in other

studies). Therefore, in this analysis, the difference in risk between the formulations was present among first-time ever users, irrespective of whether they were younger or older, had not or had been pregnant or whether they had been using the oral contraceptive for a short or long period.

First-Time Ever Use, Previous Use and Duration of Use Together

An analysis was undertaken of the WHO Collaborative Study data in which cases and controls were subdivided according to duration of use, and whether or not first time users of a particular formulation had previously used oral contraceptives.[73] Two groups were considered, desogestrel or gestodene formulation users and levonorgestrel formulation users. These were compared with non-users and the desogestrel/gestodene group was compared with levonorgestrel group. As predicted, relative to non-users, odds ratios for first-time ever users who had been using their currently-used formulation for 1 to 12 months were higher (2 to 3 times) than the odds ratios for first-time ever users who had been using their formulation for longer durations. Also in accord with the proposals outlined above, this differential between short and long term users of their currently used formulation was not apparent in those who had been using oral contraceptives prior to their currently used formulation. Also as predicted, previous users generally had lower odds ratios than first-time ever users, particularly when those who had been taking their currently used formulation for 1 to 12 months were considered. In the comparison of desogestrel/gestodene formulation users with levonorgestrel formulation users, short term, first-time ever users had an odds ratio of 2.4 (95%CI 0.5 to 11.3). The odds ratio for short term users of their currently used formulation who had taken other oral contraceptives in the past was 1.8 (0.8 to 3.8). For longer term users, the corresponding figures were 4.4 (1.1 to 17.2) and 2.3 (1.0 to 5.3). Therefore, in this analysis, the difference in risk between the formulations was maintained irrespective of whether or not a woman was a first-time user or had been using her currently used formulation for a short or long period.

Duration of Use Alone

Classification by duration of use alone was undertaken as part of the GPRD analysis. Odds ratios of 9.2 (95%CI 1.3 to 64.2) and 5.6 (0.9 to 36.3) were found for VTE in those who had been using desogestrel- and gestodene-containing formulations, respectively, for ≤6 months compared with those taking levonorgestrel-containing formulations.[28] For women who had been using oral contraceptives for more than 6 months the corresponding figures were 1.8 (0.9 to 3.5) and 1.8 (0.8 to 3.9).

With regard to differentials in risk according to age, in an analysis of data from the Transnational Study, the odds ratio for VTE among women aged 16 to 24 years was 2.6 for estrogen 0.03mg formulations containing desogestrel relative to levonorgestrel formulation users.[771] In women aged 25 to 44 years, the corresponding figure was 1.5.[53] For users of the estrogen 0.02mg formulation containing desogestrel, the pattern was reversed, the corresponding odds ratios being 0.4 among younger users and 2.8 among older users. Higher odds ratios for older users were also found for gestodene- and norgestimate-containing formulations. In this analysis, the authors argued that the older group of women was more likely to provide a balanced assessment of the effects of use of different formulations, in contrast to younger women who would have been more likely to be taking newer formulations. In the older age group, there was a regular linear relationship between increased risk of VTE and recency of introduction of a particular type of formulation. This was considered consistent with the possibility that users of older formulations represented a group from which those susceptible to VTE had been eliminated. The lack of such a relationship among younger users was taken by others as evidence against this effect.[771] [*Authors' note:* there was a 9-year age span in the younger group but a 20-year age span in the older group, so there was a greater chance of a healthy user effect becoming apparent in the older age group]. An analysis by age was also undertaken in the Leiden

Thrombophilia Study analysis.[72] Among women aged 15 to 19 years the risk of VTE for desogestrel formulation users was 7-fold higher than for levonorgestrel formulation users, whereas among women aged 20 to 24 years risk was 4-fold higher.

First-Time Use Alone

With regard to analyses with groups distinguished according to first-time use alone, in the WHO Collaborative Study analysis, there was an odds ratio of 5.2 for VTE in users of desogestrel- or gestodene-containing formulations relative to levonorgestrel formulation users among those with no history of oral contraceptive use beyond their current period of use, compared with an odds ratio of 2.1 for other current users.[85] In the first report from the Transnational Study, when only first-time users of their currently used formulation were considered, the odds ratio for VTE in users of desogestrel- or gestodene-containing formulations compared with those taking levonorgestrel- or norethisterone-containing formulations was 2.7 (95%CI 1.3 to 5.7). When only those who had used other formulations in the past were considered there was an odds ratio of 1.4 (1.0 to 2.1).[52]

Previous Pregnancy Alone

Previous pregnancy as a source of bias was considered in the Leiden Thrombophilia Study.[72] Among those who had never been pregnant, there was an odds ratio of 20.8 (95%CI 4.8 to 90.2) for DVT in desogestrel formulation users compared with non-users, whereas for levonorgestrel formulation users there was an odds ratio of 7.7 (1.8 to 32.9). The equivalent figures for those who had been pregnant were 5.1 (1.7 to 15.3) and 3.0 (1.0 to 9.6), respectively, compared with non-users.

Summary

If the biases described above were operating, it would be expected that the risk differential between desogestrel/gestodene formulation users and levonorgestrel formulation users would diminish or disappear when groups of oral contraceptive users appropriately matched for duration of use, first-time use, age or previous pregnancy were compared. In all the comparisons presented above, the risk differential remained. There was some variation in this risk differential according to duration of use, first-time use, age or previous pregnancy, however. In the PHARMO and WHO Study analyses, first-time ever, short term users (<12 months) of desogestrel/gestodene formulations had lower risks of VTE than first-time ever, longer term users, when risks relative to levonorgestrel users were considered. When the distinction was made according to first-time use alone, as opposed to first-time short versus long term, however, in both the WHO and Transnational Studies, the risk differential was greater for first-time ever users than for those who had used other formulations in the past. In the GPRD analysis, when the distinction was made according to duration of use alone, the risk differential was greater in shorter term users (≤6 months). Likewise, in the PHARMO, Transnational and Leiden Study analyses, the risk differential was greater in younger compared with older women, and in the PHARMO and Leiden Study analyses, it was greater in women who had never been pregnant compared to those who had. The generally higher risks for desogestrel/gestodene formulation users among those most likely to have been exposed to estrogens for the first time have been taken by several commentators to provide strong or conclusive support for a causal role of these formulations in the increased risk of VTE.[73,771,773]

Modelling Previous Oral Contraceptive Exposure

It should be noted that in the analyses described above, the critical underlying factor to be analysed is previous exposure to elevated estrogen levels, and to contraceptive progestogens; duration of use, first-time use, age and previous pregnancy are, in these analyses, acting as surrogate measures of previous steroid exposure. Therefore, there is some potential for confounding. For example: a long duration of previous use could include long periods during which high or low estrogen dose formulations were taken; first-time users might include a proportion of long term users who had no reason to change their formulation; and the duration of use among younger users in the analyses undertaken could have included women who had taken oral

contraceptives for up to 8 years. A somewhat different approach to adjusting for previous steroid exposure was applied in an analysis of data from the Transnational Study, in which 105 cases of VTE and 422 controls who were first time or never users of oral contraceptives were compared.[74] In this analysis, the odds ratios (adjusted for age, smoking, alcohol use, BMI and study centre) for VTE among first-time users of desogestrel/gestodene formulations compared with non-users were 14.6 (<1 year), 4.3 (1 to 2 years), 2.8 (2 to 5 years) and 4.2 (>5 years). The corresponding odds ratios for users of levonorgestrel/norgestimate formulations were 6.6, 7.2, 2.5 and 1.3. A continuous, nonlinear function of risk with duration of use was then fitted for risk of VTE in each group. The crude risk profile was about twice as high for the desogestrel/gestodene formulation users than for the levonorgestrel/norgestimate formulation users, rising to about 7 after one year of use in the former, but to only 3 in the latter. However, when the risk profiles were adjusted for age, smoking, alcohol use, BMI and study centre, the two risk profiles were superimposed throughout their course, rising to a maximum rate ratio of about 10 after 1 year of use, then to about 2 after 3 years of use. The authors noted that the convergence between the two risk profiles following adjustment was primarily due to an increase in the profile for the levonorgestrel/norgestimate formulation users and that this increase was primarily due to adjustment for age and study centre. This analysis was criticised because of the smoothed relative risk functions not being able to effectively model the instantaneous rise in risk that was presumed to occur on commencement of oral contraceptive use.[814] It was noted that when a similar approach was adopted with respect to the WHO Collaborative Study data, using smoothed functions that could accommodate an instantaneous rise in risk, the higher risk in desogestrel or gestodene formulation users compared with levonorgestrel formulation users was still apparent. In response, the Transnational Study authors criticised the WHO Collaborative Study analysis for being based on small numbers of cases and for the assumption of equivalent baseline risks in the groups being compared.[815]

Elimination of possible biases due to variation in duration of use between users of different formulations has been explored further using the Transnational Study data.[81] In this analysis, lifetime oral contraceptive exposure history was included in a Cox regression model analysis with time-dependent covariates, thus enabling adjustment of hazard ratios for variable periods of prior oral contraceptive exposure during which risk might be expected to vary. The analysis demonstrated marked differences in exposure pattern according to age and duration of use at the time the study was undertaken. Compared with non-users, the adjusted hazard ratios for VTE were as follows: formulations containing ≥0.05mg estrogen 8.48 (3.02 to 23.86); low estrogen dose formulations containing progestogens other than desogestrel or gestodene 2.85 (1.92 to 4.22); low estrogen dose formulations containing levonorgestrel 2.63 (1.75 to 3.95); norgestimate-containing formulations 3.65 (2.17 to 6.12); desogestrel- or gestodene-containing formulations 2.26 (1.46 to 3.50); and very low estrogen dose (0.02mg) formulations containing desogestrel 1.56 (0.85 to 2.86). The adjusted hazard ratio for VTE in users of desogestrel- or gestodene-containing formulations compared with low estrogen dose formulations containing other progestogens was 0.8 (0.5 to 1.3).

5.5 Anomaly of VTE Risk with Low versus Higher Estrogen Dose

In the WHO Collaborative Study, among women using a combination containing ethinylestradiol 0.02mg with desogestrel 0.15mg, there were 8 cases of VTE and only 1 control.[85] Compared with non-users, the odds ratio for VTE among desogestrel combination users were 7.6 (3.9 to 14.7) for users of the 0.03mg estrogen dose formulation and 38.2 (4.5 to 325) for the 0.02mg formulation. A similar inversion of the expected pattern of dose response relationship was found among users of formulations containing the antiandrogenic progestogen, cyproterone. In the GPRD analysis the

crude odds ratios for users of formulations containing desogestrel 0.15mg in combination with either 0.02mg (4 cases, 9 controls exposed) or 0.03mg (26 cases, 82 controls exposed) estrogen were 2.7 and 1.9, respectively,[28] although in a recent analysis of data from the GPRD database covering the period 1992 to 1997, and with close matching for age between cases and controls, Farmer and colleagues found odds ratios of 0.8 and 1.0 for the very low and low estrogen dose desogestrel formulations.[793] According to the Transnational Study, the odds ratios for the very low and low estrogen dose desogestrel formulations were 1.6 (15 cases, 36 controls exposed) and 1.9 (64 cases, 110 controls exposed), respectively.[53] However, when odds ratios among women aged 16 to 24 and 25 to 44 years were considered separately, the corresponding figures for the younger women were: very low dose 0.4 (2 cases, 20 controls exposed); and low dose 2.8 (32 cases, 48 controls exposed). For the older women, the odds ratios for the very low and low dose formulations were 2.8 (13 cases, 16 controls exposed) and 1.5 (32 cases, 62 controls exposed), respectively. In the MediPlus database nested case-control analysis the adjusted odds ratio for VTE among users of the estrogen 0.02mg dose desogestrel formulation compared with users of levonorgestrel or norethisterone formulations was 3.49 (95%CI 1.21 to 10.12) whereas the corresponding figure for users of the estrogen 0.03mg dose formulations containing desogestrel or gestodene was 1.18 (0.66 to 2.17).[86] However, in a subsequent analysis of the MediPlus database, extending between 1992 and 1997, and with a greater number of cases and somewhat more rigorous criteria for inclusion, these differentials disappeared:[791] adjusted odds ratios of 1.1 (95%CI 0.5 to 2.6) and 1.1 (0.4 to 3.4) were found for low and very low estrogen dose desogestrel formulation users, respectively, relative to low estrogen dose levonorgestrel formulation users. An increased odds ratio in users of the very low estrogen dose desogestrel formulation was also seen in the study of Bloemenkamp et al.[36] For the formulation containing ethinylestradiol 0.03mg (22 cases, 29 controls), the odds ratio (adjusted for age, family history of venous thrombosis, calender time and centre) was 4.9 (2.5 to 9.4), whereas for the equivalent formulation containing ethinylestradiol 0.02mg (6 cases, 1 control) the odds ratio was 24.7 (2.8 to 213.5).

The interpretation of these anomalous relationships has been that they demonstrate the likely operation of prescribing bias, with the very low estrogen dose formulations being prescribed to women at greater risk of a thrombosis. It was noteworthy that in the MediPlus analysis, the age distribution of users of the 0.02mg estrogen/desogestrel formulation was markedly shifted towards higher age groups, compared with users of other combined formulations.[86] However, in this analysis, age adjustment did not eliminate the excess risk for this formulation. With regard to the study of Bloemenkamp et al.,[36] the authors considered that the risk profile of users of the very low estrogen dose formulation was very similar to that of other oral contraceptive users and concluded that preferential prescribing could not entirely explain the high odds ratio.

The only analysis that would accord with the expected relative reduction in risk of VTE in users of the very low estrogen dose formulation compared with users of low dose formulations was that of the Transnational Study data by Lewis et al. in which full exposure was entered into a Cox regression model.[81] After this adjustment, low estrogen dose desogestrel- or gestodene-containing formulations were associated with a hazard ratio of 2.26 (1.46 to 3.50), whereas with the very low estrogen dose (0.02mg) formulations containing desogestrel the hazard ratio was 1.56 (0.85 to 2.86).

5.6 Differences in Risk of Arterial Disease

In the early 1980s, the possibility was raised that risk of arterial disease associated with oral contraceptive use might be due to a progestogen-related lowering of HDL levels.[135] One justification for promoting desogestrel and gestodene as new progestogens was the observation that, in low estrogen dose formulations, their use was associated with an increase in HDL levels compared with the reduc-

tions or lack of effect seen with oral contraceptives containing other progestogens. Given the manifold properties of HDL which would accord with vascular protection, and evidence from studies demonstrating reduced vascular disease in response to the raising of HDL levels, there were grounds for supposing that use of oral contraceptive formulations that raised HDL would be associated with reduced arterial disease relative to use of formulations which did not affect HDL levels, or lowered them.[772]

5.6.1 MI

The WHO Collaborative Study findings on MI were based on 388 cases of MI and 941 controls.[100] There were 3 cases and 5 matched controls using desogestrel or gestodene formulations and their estimated risk compared with non users (age-matched and adjusted for cigarette smoking) was 0.97 (0.14 to 6.96). The equivalent figure for the 13 cases and 17 controls taking levonorgestrel-containing formulations was 1.64 (0.49 to 5.54). It was stated that risk estimates for women who had reported receiving a BP check were identical between users of low estrogen dose desogestrel or gestodene formulations and levonorgestrel formulations. In other words, users of desogestrel or gestodene oral contraceptives were indeed less likely to experience a MI than those using other formulations, but, rather than being a consequence of the characteristics of the progestogen they were using, this could be explained by the quality of their healthcare or by more rigorous exclusion from oral contraceptive use of women with high BP.

A GPRD nested case-control analysis (4 controls per case) of fatal or nonfatal MI was undertaken involving 303 470 women who had taken oral contraceptives between January 1991 and October 1994.[816] There were 11 cases of MI, 7 among current users of oral contraceptives. Relative risk compared with users of levonorgestrel formulations was 0.7 (95%CI 0.1 to 8.2) for desogestrel formulation users and 0.6 (0.1 to 6.4) for gestodene formulation users.[816]

In the first report from the Transnational Study on risk of MI among users of different low estrogen dose formulations, 153 cases of fatal or nonfatal MI were compared with 498 controls (both hospital and community based and matched within 5-year age bands) from 16 centres in Europe; there were 6 cases and 34 controls exposed to desogestrel- or gestodene-containing formulations.[119] In a subsequent analysis, undertaken after recruitment for the study had ceased, 182 cases were compared with 635 controls, with 7 cases and 49 controls exposed to desogestrel- or gestodene-containing formulations.[122,123] In accord with the claims made on the basis of biochemical effects of these formulations, the odds ratios (adjusted for study centre, age, BMI, smoking, alcohol use and duration of oral contraceptive use) for MI in users of desogestrel- or gestodene-containing formulations compared with those taking predominantly levonorgestrel-containing formulations were, for the first analysis 0.36 (0.1 to 1.2) and for the second analysis 0.28 (0.09 to 0.86). This relative protection was only apparent with community controls or in non-UK study centres. BP checking made little difference to these estimates, the crude odds ratio for MI among users of desogestrel or gestodene formulations compared with those using predominantly levonorgestrel formulations being 0.37 (0.15 to 0.91) without adjustment for BP checking and 0.27 (0.10 to 0.80) with adjustment (personal communication; M.A. Lewis, 2000).

In the first analysis of risk of MI from the Transnational Study, crude odds ratios for MI among smokers compared with nonsmokers were 3.1 (0.5 to 19.8) for users of low estrogen dose formulations containing desogestrel or gestodene, 7.7 (4.0 to 14.7) for women not using oral contraceptives, and 11.1 (3.0 to 40.2) for users of low estrogen dose formulations containing mainly levonorgestrel.[119] In the second analysis, the corresponding figures were 3.8, 7.2 and 9.5.[122] With stratification for age, crude odds ratios for risk of MI among users of desogestrel or gestodene formulations compared with users of formulations containing mainly levonorgestrel were 0.71 (0.22 to 2.39) for

women aged 25 to 34 years and 0.20 (0.04 to 0.93) for women aged 35 to 44 years.[122] These findings suggest that there was relative protection from the risks of arterial disease associated with smoking and aging among users of desogestrel- or gestodene-containing formulations.

In contrast to the WHO Collaborative Study, there was no difference in risk of MI in the Transnational Study when nonsmoking oral contraceptive users were compared with nonsmoking nonusers (OR 1.32: 95% CI 0.51 to 3.45).[122] In the WHO Collaborative Study, the increased risk associated with oral contraceptive use among nonsmokers disappeared when previous BP checking was taken into account.[100] As mentioned, there was relatively little effect of previous BP checking on MI risk in the Transnational Study (odds ratio for women who had not had a previous BP measurement compared with those who had: 1.81; 95% CI 1.20 to 2.73).[122] Thus, in the WHO Collaborative Study, the increased risk of MI associated with oral contraceptive use in nonsmokers could be accounted for by lack of BP checking. In the Transnational Study, there was little increased risk of MI with oral contraceptive use among nonsmokers and little effect of BP checking.

The relative absence of any effect of BP checking in the Transnational Study was also apparent in comparing odds ratios for MI according to BP checking and oral contraceptive use. Among those who had not received a BP check in the Transnational Study, the odds ratio for MI for oral contraceptive use compared to non-use was 2.8 (1.4 to 5.6), whereas among those who had received a BP check the corresponding figure was 1.1 (0.7 to 1.7).[122] For the WHO Collaborative Study there was a much greater differential, the corresponding odds ratios being 9.5 and 2.6.[100] In the Transnational Study, 13% of cases and 6% of controls had missing information with regard to previous BP measurement.

With follow-up of cases identified using the Danish Hospital Register system, Lidegaard and colleagues[29,817] identified 88 women aged 15 to 44 years who had experienced a MI, with no known predisposing disease, in the period 1994 to 1995. 1045 controls were available for comparison. The adjusted odds ratio for MI among users of oral contraceptives containing ethinylestradiol 0.05mg and an estrane progestogen was 4.1 (95%CI 1.6 to 10.9). For users of formulations containing levonorgestrel or norgestimate, the odds ratio was 1.9 (0.7 to 4.9) and for users of desogestrel/gestodene formulations 1.1 (0.5 to 2.5). Among the users of desogestrel/gestodene formulations, the odds ratio decreased with increasing duration of use, being 1.9 (0.4 to 9.2), 0.5 (0.1 to 3.9) and 0.8 (0.2 to 2.7) for the first year of use, 1 to 5 years of use and more than 5 years of use, respectively. Odds ratios for acute MI among users of low estrogen dose formulations containing levonorgestrel or norgestimate were 3.3, 7.8 and 11.5 with increasing number of cigarettes smoked (1-10, 11-20, >20/day, respectively), whereas for desogestrel or gestodene formulations the odds ratios were 2.0, 4.8 and 7.0.[818]

In the MICA Study, initiated in responce to findings from the Transnational and WHO Collaborative Studies, 448 cases of fatal or nonfatal MI occurring between October 1993 and October 1995 were compared with 1728 community-based controls.[121] The odds ratios (adjusted for cigarette smoking, diabetes mellitus, family history of ischaemic heart disease, other drugs taken in the past year, BMI, history of hypertension or angina pectoris, and recent BP measurement) for MI among users of levonorgestrel or norethisterone formulations was 1.10 (95%CI 0.52 to 2.30) and among users of desogestrel or gestodene formulations 1.96 (0.87 to 4.39). No information was given with regard to risk differentials between different formulations according to BP checking or cigarette smoking.

5.6.2 Stroke

Risk of ischaemic stroke in the WHO Collaborative Study was evaluated in 697 cases and 1962 age-matched hospital controls.[177] In Europe, the odds ratio (adjusted for history of hypertension, number of live births and smoking) for ischaemic stroke for users of low estrogen dose levonorgestrel-containing formulations (15 cases, 44 controls ex-

posed) was 1.36 (95%CI 0.60 to 3.07), whereas for users of desogestrel/gestodene formulations (4 cases, 8 controls exposed) the odds ratio was 1.76 (0.33 to 9.36). In developing countries, the odds ratio (adjusted for history of hypertension or rheumatic heart disease, and smoking) for ischaemic stroke for users of low estrogen dose levonorgestrel-containing formulations (55 cases, 79 controls exposed) was 3.39 (2.24 to 5.13), whereas for users of desogestrel- or gestodene-containing formulations (4 cases, 7 controls exposed) the odds ratio was 1.18 (0.24 to 5.86). Data from the developing country centres was analysed according to whether or not women had received a BP check before they started using oral contraceptives. Among low-estrogen dose levonorgestrel-containing formulation users, the odds ratio (adjusted for history of hypertension or rheumatic heart disease, and smoking) for those who had received a BP check (21 cases, 45 controls exposed) was 2.05 (1.12 to 3.77) and for those who had not received a BP check (34 cases, 34 controls exposed) 5.16 (2.92 to 9.12). Among the European centres, there was little distinction in risk according to whether BP had been checked or not. With regard to haemorrhagic stroke, the WHO Collaborative Study compared 1068 cases with 2910 age-matched controls and found no difference in risk according to the type of progestogen used.[190]

Data on stroke and oral contraceptive use from the WHO Collaborative Study was subsequently examined in more detail for differences in odds ratios between users of different types of oral contraceptive.[819] Among all women for whom details of oral contraceptive use were known, there were 1455 cases of stroke (489 ischaemic, 715 haemorrhagic and 251 unspecified) and 3967 hospital controls. Among users of desogestrel or gestodene formulations, the odds ratio for ischaemic stroke were 1.75 (0.59 to 5.16), whereas among users of levonorgestrel formulations the odds ratio was 2.70 (1.77 to 4.14). Among women who reported having had a BP check, the odds ratios were 1.63 (0.43 to 6.61) and 1.95 (1.06 to 3.58), respectively. For haemorrhagic stroke, odds ratios were 1.70 (0.72 to 4.02) among users of desogestrel or gestodene formulations and 1.73 (1.19 to 2.53) among levonorgestrel formulation users. Haemorrhagic stroke was also examined in a nested case-control analysis of data from the GPRD Study.[820] Age-adjusted relative risks of 1.2 (0.2 to 6.8) and 1.4 (0.2 to 8.2) were found for desogestrel and gestodene formulation users, respectively, compared with users of other formulations.

The Transnational Study analysis of thromboembolic stroke compared 220 cases with 775 hospital or community-based controls.[182] The matched odds ratio (adjusted for hypertension, smoking and number of births) for thromboembolic stroke for desogestrel/gestodene formulation users compared with levonorgestrel formulation users was 1.4 (0.8 to 2.4).

With follow-up of cases identified using the Danish Hospital Register system, Lidegaard and colleagues[31] identified 219 women aged 15 to 44 years who had experienced a cerebral thromboembolism, with no known predisposing factors, in the period 1994 to 1995. 1041 controls were available for comparison. The odds ratio for cerebral thromboembolism in users of desogestrel/gestodene formulations was 1.3 (0.8 to 2.2) and for users of levonorgestrel/norgestimate formulations 2.4 (1.4 to 4.2).[183]

5.7 Possible Biological Mechanisms

Controversy over the issue of biological plausibility centres on the question: can the observed differences in risk of VTE associated with use of different low estrogen dose oral contraceptives be explained by confounding and bias, or are they a direct consequence of use of the particular formulations concerned? Issues of confounding and bias have been outlined in section 5.4. With regard to there being direct and differing effects of the formulations concerned, convincing support for this possibility would come from a demonstration of the mechanism for oral contraceptive-induced VTE, and a further demonstration that this mechanism was differently affected by the different formulations in a way that accorded with observed differ-

ences in risk of VTE. In this respect, there have been two basic suppositions. The first, which was first raised by the authors of the WHO Collaborative Study report,[32] and subsequently by others,[821] is that risk of thrombosis is conferred by the estrogen component of the combined oral contraceptive. Differences in risk according to progestogen content then arise because of differences in the extent to which the progestogen opposes the prothrombotic effects of the estrogen. In accord with this, desogestrel and gestodene were known to be less anti-estrogenic than levonorgestrel with respect to effects on metabolic variables such as sex hormone binding globulin, serum triglycerides or HDL cholesterol. The other proposal was that the increased risk of thrombosis was conferred by an unexpected property of the new progestogens, desogestrel and gestodene.[36,773,822,823] Although there is no precedent for the latter proposal, neither of these possibilities can be excluded as long as a definitive mechanism for oral contraceptive-induced VTE eludes investigators.

The principal programme of research into the mechanism for oral contraceptive-induced VTE has concerned disturbances in factors of the haemostatic system, and it has been in this area that a mechanistic basis for the differences in risk of VTE between users of different low estrogen dose oral contraceptives has been sought. Prior to the report from the WHO Collaborative Study, the few trials undertaken in which low estrogen dose desogestrel or gestodene formulations were compared with equivalent levonorgestrel formulations had identified few significant differences with the exception of FVII, which was increased by about 25% in users of desogestrel or gestodene formulations compared with a rise of about 5% among levonorgestrel formulation users.[749]

The questions raised by publication of the WHO Collaborative Study findings initiated several randomised trials of the effects of low estrogen dose formulations differing in progestogen content on a broad range of factors, activities and tests of the function of the haemostatic system. At the time of writing, not all of these have been completed and many of the results from those that have are only available in abstract form. However, the dominant track of investigation over the past two years has concerned the observation by Rosing et al.[697] of a marked difference in the sensitivity to APC between users of different formulations that accorded with the differences reported in risk of VTE. This observation has itself generated an appreciable programme of research in different centres.

5.7.1 Activated Protein C Resistance

The nature and importance of APC resistance has been described in section 4.9.3. With regard to differing effects of different low estrogen dose oral contraceptives on the haemostatic system, the core observation concerns Rosing and colleagues' study,[697] published in 1997, of APC resistance measured as the normalised APC sensitivity ratio using the EP/ETP method, with thrombin generation initiated via the extrinsic pathway and measured as the endogenous thrombin potential. Using this measure, significantly greater resistance to APC was apparent in users of low estrogen dose desogestrel-containing formulations compared with identical estrogen dose levonorgestrel-containing formulations [no oral contraceptive (OC) 1.22, triphasic OC 1.93, 'second generation' OC 1.81, 'third generation' OC 2.59]. These differences were independent of variation in protein S and were apparent in women within 3 days of commencing oral contraceptive use. The authors conjectured that the differences in APC sensitivity ratio between users of desogestrel- and levonorgestrel- or norethisterone-containing formulations were due to a property of the progestogen. They also noted that 5 oral contraceptive users who were heterozygous for the factor V Leiden mutation had a mean APC sensitivity ratio that equalled the sum of the mean ratio seen in the desogestrel formulation users and in carriers of the mutation. This suggested that oral contraceptives could decrease APC sensitivity by an effect that was independent of the level at which the factor V Leiden mutation was operating, but which did impinge at some level on the extrinsic pathway. The effects of the factor V Leiden mutation had already established the prin-

ciple that APC resistance could confer increased risk of VTE, and Rosing's observations[697] were immediately hailed as having demonstrated the biological plausibility of the increased risk of VTE in users of desogestrel- and (by implication) gestodene-containing formulations.[823]

Initial criticisms of the observation of Rosing et al.[697] centred around the need for confirmation, and for randomised study groups.[824] It was also noted that there was no difference between older and newer formulations when the APC sensitivity ratio was estimated with thrombin generation triggered via the intrinsic pathway and estimated as the aPTT. Thus, Olivieri et al.,[825] observed that, among 50 oral contraceptive users, variation in APC sensitivity ratio was not associated with duration of OC use, steroid dose or type of formulation and, in the Bavarian Thromboembolic Risk Study (BATERS), a 12% lower APC sensitivity was found in oral contraceptive users but, again there was no difference between different oral contraceptive formulations.[690,754]

With regard to confirmation of Rosing and colleagues' observation, Kluft et al.[756] examined variation in EP/ETP-based APC sensitivity ratio measures of APC resistance with oral contraceptive progestogen content in the BATERS study. Among 30 users of monophasic desogestrel/gestodene combined formulations APC sensitivity ratio was 2.6, whereas among 21 monophasic levonorgestrel formulation users the ratio was 1.3. Among 35 users of multiphasic levonorgestrel-containing formulations, with a lower overall progestogen dose, the ratio was increased to 1.7. The ratio showed a significant negative correlation with levonorgestrel dose ($r = -0.37$, $p = 0.005$, $n = 57$). Variation with oral contraceptive formulation in APC sensitivity ratio measured using the EP/ETP-based pathway was also confirmed by Thomassen et al.[757] The ratio was 1.35 in 62 women not taking oral contraceptives, 2.21 among 62 users of 'second generation' oral contraceptives, 2.87 among 64 users of 'third generation' formulations, 3.43 among 26 factor V Leiden positive women not taking oral contraceptives and 4.15 among 30 pregnant women. Confirmation of Rosing's original 1997 report on the effects of oral contraceptives on the normalised APC sensitivity ratio using the EP/ETP-based assay has recently come from Rosing's own group.[758] In a randomised cross-over study (2-month washout) of 28 women comparing the effects of levonorgestrel 0.15mg or desogestrel 0.15mg both combined ethinylestradiol 0.03mg, the normalised APC sensitivity ratio significantly increased during oral contraceptive use. The increase was significantly greater among users of the desogestrel formulation than the levonorgestrel formulation.

The differences in variation in oral contraceptive-associated APC resistance apparent in EP/ETP and aPTT assays were explored in a comparative study by Curvers et al.[755] In this study, the original finding by Rosing et al. was confirmed. In addition, significantly lower APC sensitivity was found in oral contraceptive users when using the aPTT-based assay, but there was no difference between formulations. Both assays discriminated the presence of factor V Leiden mutation equally well. They also discriminated the joint effect of factor V Leiden with oral contraceptive use on APC sensitivity, although there was somewhat greater discrimination using the EP/ETP-based assay. The APC sensitivity ratio measured by the two assays correlated in non users of oral contraceptives but not in users. The authors concluded that oral contraceptives must change the level of unidentified plasma protein components that modulate the effect of APC on thrombin formation initiated via the extrinsic pathway.

Authors' note: at least 10 different assays for measurement of the APC sensitivity ratio may be distinguished, according to the means by which coagulation or thrombin generation is triggered. With variation according to sample handling, reaction concentrations and dilution with factor V-deficient plasma, this number multiplies considerably. The ability of the different assays to distinguish different states of hypercoagulability differs considerably. For example, complete discrimination for factor V Leiden status may be achieved, but with

no discrimination of pregnancy and oral contraceptive use.[826] There is, therefore, uncertainty about the clinical relevance of assays that discriminate acquired APC resistance and the extent to which they are simply providing surrogate measures for variation in the concentration or activity of the various procoagujant or anticoagulant factors that affect APC sensitivity.

5.7.2 Other Haemostatic System Measures

As reported in a recent series of papers, the effects of levonorgestrel 0.15mg or desogestrel 0.15mg both combined with ethinylestradiol 0.03mg on haemostatic factors were compared in a randomised cross-over trial (2-month washout) of 28 women. Factors II, VII, X and fibrinogen increased significantly in response to levonorgestrel or desogestrel formulations (12 of 16%, 12 of 32%, 22 of 25% and 0.5 of 0.5 g/L, respectively).[827] Factor V decreased by 11% among the desogestrel formulation users. The increases in factors II and VII and decrease in factor V were significantly greater among the desogestrel formulation users. F_{1+2} increased significantly, with no difference between formulations. TAT complex and soluble fibrin concentrations did not change. t-PA activity, plasminogen, PAP and D-dimer increased significantly and PAI-1 antigen, PAI-1 activity and t-PA antigen decreased significantly.[828] There were no differences between formulations in these changes. An increase in thrombin-activatable fibrinolysis inhibitor (TAFI) levels was greater in desogestrel compared with levonorgestrel formulation users (an increase in TAFI levels suggests a decrease in fibrinolytic potential). Clot lysis times were expressed as a ratio in the absence and presence of a blocking antifactor XI antibody. Clot lysis times with blocking of factor XI were prolonged with oral contraceptive use, but there was no detectable effect without blocking of factor XI. Clot lysis time depends on the generation of thrombin via the intrinsic pathway in a factor dependent manner. These findings suggest that more thrombin is generated in the presence of oral contraceptives in a factor XI-independent manner. In accord with this, oral contraceptives significantly increased the generation of F_{1+2} during clot formation. This meant that in response to oral contraceptives, F_{1+2} generation in the clot is less dependent on factor XI. This factor XI-independent F_{1+2} generation was greater with the desogestrel formulation than with the levonorgestrel formulation. This trial also included a detailed evaluation of factors and activities of the anticoagulant pathways.[829] Use of the desogestrel-containing combination was associated with significant increases in plasma levels of α2-macroglobulin, α1-antrypsin, protein C inhibitor and protein C, and significant decreases in antithrombin and protein S. Lesser, nonsignificant changes, were seen during use of the levonorgestrel-containing combination. Relative to the levonorgestrel formulation, use of the desogestrel formulation was associated with a significantly greater fall in protein S and greater APC resistance. The latter was apparent in a 5% fall with the intrinsic pathway/aPTT assay, compared with no change in the levonorgestrel formulation users, and a 92% rise with the EP/ETP assay compared with a 56% rise in the levonorgestrel formulation users. The increased APC resistance apparent in the EP/ETP measures correlated with the change in protein S on treatment.

At the time of writing (August 2000), further information on the comparative effects of different progestogens in combined oral contraceptives on the haemostatic system is expected from a large (n = 730) randomised trial of 7 different formulations.[830] Principal outcome variables are F_{1+2} and D-dimer, and provision is made in the study design for evaluation of the effects of both progestogen type (levonorgestrel, desogestrel, gestodene and norgestimate) and estrogen dose (0.050, 0.035, 0.030 and 0.020mg ethinylestradiol).

In summary, the principal differences in measures of the haemostatic system between users of desogestrel- or gestodene-containing formulations compared with levonorgestrel-containing formulations are higher FVII levels, greater APC resistance as measured by the EP/ETP method, but not by the intrinsic pathway/APTT method, (excepting the 5% increase detected by Tans and colleagues[829])

and a greater factor XI-independent F_{1+2} generation.

5.7.3 Possible Clinical Significance

Consideration of the possible clinical significance of the differences described in sections 5.7.1 and 2 has centred on the associations between haemostatic factors and activity measures and VTE in case-control studies of risk factors for VTE. Thus, if low estrogen dose desogestrel- or gestodene-containing formulations have a greater effect on a factor or activity measure than do low estrogen dose levonorgestrel-containing formulations, and, in epidemiological studies, such variation in the factor or activity measure is associated with increased risk of VTE, then the biological plausibility of a direct effect of the formulations concerned is strengthened. As described in section 5.7.2, the measures to be considered are higher FVII levels, greater APC resistance (as measured by the EP/ETP method, but not by the intrinsic pathway/aPTT method), and a greater factor XI-independent F_{1+2} generation.

As described in section 4.9.3, in the Leiden Thrombophilia Study, increased FVII levels were not associated with increased risk of VTE.[719] In addition, the association between the factor V Leiden mutation and APC resistance established the principle that APC resistance could provide an index of thrombotic risk. However, APC resistance can be measured in a number of different ways, each of which can provide different degrees of discrimination according to the condition in which the haemostatic system is affected. Such conditions include the presence of factor V Leiden mutation, but also include conditions of acquired APC resistance in which the resistance may be due to changes in factors of the intrinsic or extrinsic coagulation pathways. Studies of association between different measures of APC resistance and VTE are described in section 4.9.3. In brief, the weight of evidence indicates that APC resistance measured using the intrinsic pathway/aPTT method is associated with VTE.[702] It is noteworthy, in this respect, that when current oral contraceptive users who had experienced a previous thrombotic event were compared with oral contraceptive users who had remained healthy, the only significant difference in haemostatic measures was an increased APC resistance, measured using the intrinsic pathway/aPTT method, in those who had experienced a thrombotic event.[703] In accord with the observation that an increase in intrinsic pathway FVIII is associated with VTE,[764] some of the association between APC resistance, measured by the intrinsic pathway/aPTT method, and VTE could be explained by variation in FVIII levels.[702] However, there is little evidence for a difference between different low estrogen dose oral contraceptive formulations in FVIII or in APC resistance measured by the intrinsic pathway/aPTT method. So, although variation in these measures with oral contraceptive use might contribute to oral contraceptive-induced VTE in general, such variation has yet to provide a convincing biological basis for variation in risk according to oral contraceptive composition.

Evidence regarding the clinical significance of APC resistance measured using the EP/ETP assay is more equivocal with one study in women showing no association[699] and one in men showing an association.[700] Moreover, to date, no associations have been found between factors of the extrinsic pathway (which may be responsible for variation according to oral contraceptive composition in APC resistance measured using the EP/ETP method) and VTE. Therefore, it remains questionable whether APC resistance measured using the EP/ETP assay provides a biological basis for variation in risk according to oral contraceptive composition.

With regard to the one other haemostatic difference so far identified between users of low estrogen dose oral contraceptives containing desogestrel or levonorgestrel, elevated TAFI levels are a mild risk factor for venous thrombosis,[831] the clinical significance of an increase in factor XI-independent prothrombin F_{1+2} generation is at present unknown.

At the time of completing this work, the findings of a study have been reported which shows increased thrombin receptor expression in rat aortic smooth muscle cells in response to etonogestrel

(3-ketodesogestrel), gestodene, medroxyprogesterone and progesterone.[832] This effect was not seen with levonorgestrel, norethisterone, norgestimate or 17α-ethinylestradiol. This study raises the possibility that progestogen-specific effects at the cellular level on elements of the haemostatic system do exist. However, confirmation is necessary and, if confirmed, many questions will need to answered before the clinical significance of this observation is elucidated. For example, is there evidence *in vivo* for consistent differentials in thrombin receptor activation according to progestogen type in, say, prostaglandin release or platelet aggregability?

5.8 Conclusions

Published matched, adjusted odds ratios for risk of VTE among users of low estrogen dose desogestrel or gestodene formulations compared with levonorgestrel formulations have varied between 0.6 and 4.2. If the odds ratio between the two types of formulation is taken as 2 and this epidemiological evidence is interpreted causally, the excess risk for VTE caused by use of desogestrel- or gestodene-containing formulations compared with levonorgestrel-containing formulations is 15 extra cases of VTE per 100 000 women per year, against reference risks of about 10 to 15 cases per 100 000 women per year for users of levonorgestrel and 3 to 5 per 100 000 women per year for women not taking oral contraceptives.[28,85,833]

The strongest evidence for the formulations in question causing this risk difference comes from the comparisons which show its presence in first-time ever, short term users of oral contraceptives. However, it should be borne in mind that much of this evidence comes from periods of prescribing when desogestrel- and gestodene-containing formulations were perceived as being safer with respect to risk of VTE and may, therefore, have been preferentially prescribed to women already at some risk. The strongest evidence that the difference in risk between different formulations is not caused by the steroids in question, but is rather a consequence of bias, comes from the analysis from the Transnational Study in which the difference was eliminated by incorporation of full exposure history into the analysis. However, whether such adjustment can completely eliminate the risk differentials in first-time ever, short term users of oral contraceptives needs to be confirmed. Any new information on these risk differentials is now inevitably going to be influenced by the controversy that has continued since 1995. A number of countries now require the package insert to carry a warning. Further analyses and studies in this area should, nevertheless, continue, with particular care being taken to distinguish as clearly as possible the magnitude and duration of previous exposure to contraceptive steroids, and to identify as accurately as possible how such past exposure can affect risk estimates. This latter requirement has barely been met in the studies so far undertaken.

The strongest evidence overall that there are biases at work in these studies comes from the consistently higher odds ratios for VTE among users of very low estrogen dose desogestrel-containing formulations compared with low estrogen dose formulations. To account for this without invoking bias it would be necessary to propose an inverse dose response relationship between estrogen and VTE, or some unique 'third generation effect'. The latter would require desogestrel to have thrombotic properties that are opposed by ethinylestradiol. In the absence of an agreed mechanism for oral contraceptive-induced VTE, it must be acknowledged that neither of these possibilities are excluded. However, since both require a complete inversion of currently held opinion regarding the prothrombotic effects of estrogens, the simplest interpretation is that these differentials reflect the operation of bias, with such very low dose formulations being preferentially prescribed to women perceived to be at increased risk of VTE. It is particularly unfortunate that such very low dose formulations are no longer more widely prescribed, since the justification for caution in their general use seems especially tenuous, and important information has yet to be gathered on their long term effects during more general use.

With regard to possible differentials in arterial disease risk, evidence remains equivocal. Risk of MI was significantly reduced among users of desogestrel or gestodene formulations compared with levonorgestrel formulation users in one study, but there was no difference in 4 others. One of the negative studies returned lower odds ratios for MI in users of desogestrel or gestodene formulations, but numbers were very limited.[816]

One of the more striking findings with regard to arterial disease has been the powerful influence of previous BP measurement on risk estimates in the WHO Collaborative Study. Information on this variable was originally gathered in order to provide an index of access to healthcare facilities. As described in section 4.5.2, it is unclear what this information might actually mean, and the WHO Collaborative Study authors indeed questioned whether the lower risks for MI among oral contraceptive users who had reported a BP check before their current episode of oral contraceptive use reflected screening for increased BP or other aspects of health care or healthcare-seeking behaviour.[100] Further questions might also be raised. For example, what proportion of women do not commence use of oral contraceptives because of the results of BP checking? What proportion of oral contraceptive users cease to use oral contraceptives because of adverse changes in BP? Are there differences between different formulations with respect to cessation of use because of elevated BP? Might BP checking differentially affect arterial disease risk among users of different oral contraceptives formulations? In this respect, the consistent finding of higher BP associated with use of levonorgestrel-containing formulations (see section 4.5.2) may be important. If a levonorgestrel-induced increase in BP increases arterial disease risk, then any increased risks of arterial disease among levonorgestrel formulation users might be expected to be reduced by including in the analysis only those who have had a BP check, in other words, by screening out those with undetected high BP.

These findings from the WHO Collaborative Study with regard to BP checking, and from the Transnational Study with regard to the lack of risk in nonsmoking oral contraceptive users, strengthen the possibility that if all risk factors for arterial disease are eliminated there is no effect of oral contraceptive use on arterial disease incidence. This, and the marked synergistic effects that appear when oral contraceptive use is combined with arterial disease risk factors, argues in favour of a model of arterial disease in oral contraceptive users that combines a subclinical cycle of endothelial damage and repair with the intervention of the prothrombotic effects of oral contraceptives. In the absence of arterial occlusion, the endothelial damage associated with risk factors such as smoking or hypertension is effectively countered by arterial repair mechanisms, and oral contraceptives alone are insufficient to cause arterial occlusion. In combination, however, the effects of contraceptive steroids rapidly overwhelm the repair mechanisms.

If such mechanisms were indeed responsible for increased risk of arterial disease in oral contraceptive users, it would then not be surprising if any differences in arterial disease risk between users of low estrogen dose desogestrel or gestodene formulations and levonorgestrel formulations were to diminish as underlying arterial disease risk, and the accompanying endothelial damage, is progressively eliminated from the groups under consideration. Consequently, the real issue is not what risk of arterial disease is associated with one particular formulation compared with another, but instead, what is the influence of a particular formulation on the effects of established risk factors? Analyses from this perspective were undertaken in the course of the Transnational Study with regard to cigarette smoking and aging, and suggested that the anticipated protective effect of desogestrel or gestodene formulations was present. With these underlying issues now better clarified, an analysis of the WHO Collaborative Study and Transnational Study MI datasets in combination could be particularly useful. With the larger numbers of cases thus available, such issues as the influence of BP checking in different groups of oral contraceptive users might be somewhat better clarified, and the possi-

bility of relative protection or exacerbation afforded by the different formulations in relation to the effects of aging, cigarette smoking or undiagnosed hypertension more effectively explored. In general, it would be preferable for women to take oral contraceptives only if they have no risk factors whatsoever for arterial disease. However, many who wish to take oral contraceptives do have such risk factors and choose the increased risk rather than resort to other forms of contraception. More knowledge of how they are likely to be affected would be of value.

With regard to the biological plausibility of differentials in arterial disease risk, the newer oral contraceptives are characterised by their ability to raise HDL cholesterol levels and, as mentioned above, this property was nominated as a beneficial effect of these formulations. As described in previous sections, evidence in favour of the benefits of raising HDL is now becoming compelling[427,428] and recommends further investigation into the clinical significance of oral contraceptive-induced increases in HDL.

Issues of biological plausibility with regard to VTE risk differentials among users of different oral contraceptives are considered last in this section because this is the area in which there is currently the most intensive activity and in which current summaries are most likely to be rapidly superseded. There is no doubt that low estrogen desogestrel- or gestodene-containing formulations differ from equivalent levonorgestrel-containing formulations in their effects on metabolic and haemostatic systems. The question is, do these differences have any clinical significance? It is important to note that this question must be answered independently of any supposed differences between the formulations in their effects on risk of VTE. Otherwise, circular lines of reasoning can easily develop whereby the supposed difference in risk is taken as evidence of the importance of an observed difference in a particular haemostatic measure, which difference is then taken to confirm the supposed difference in risk. At present, there are two confirmed differences between low estrogen dose desogestrel or gestodene formulations and equivalent levonorgestrel formulations, namely increased FVII levels and increased APC resistance measured with thrombin generation evaluated as the endogenous thrombin potential and initiated via the extrinsic pathway. Future publication of results from current randomised trials may be expected to confirm these differences and provide more rigorous comparisons of the effects of different progestogens and estrogen doses. Further differences may emerge with the broader range of haemostatic measures currently being considered. Beyond this, the changes responsible for the increased APC resistance need to be identified. At present, FVII and TFPI seem the most likely candidates. The clinical significance of changes in APC sensitivity measured by the EP/ETP method, and of any accompanying changes there may be in fibrin generation or fibrinolysis, remains to be established. Given the complexity of the changes in the haemostatic system in oral contraceptive users, and, presumably, the transient variations in concentration and activity that may occur, insight into mechanisms responsible for oral contraceptive-induced VTE may only come from prospective studies with long term follow-up of variation in haemostatic measures in oral contraceptive users. It is appreciated that, at present, the necessary study would be prohibitively expensive. It would also be of questionable value given how much remains to be clarified concerning the effects of oral contraceptives on the haemostatic system. However, with greater insight into the workings of the haemostatic system and the effects of oral contraceptives, at least the potential value of such a prospective study could increase considerably.

6. Conclusions

Women taking the combined oral contraceptives in current use have a risk for VTE of between 15 and 45 cases per 100 000 women per year compared with about 5 to 10 cases per 100 000 women per year in comparable women not taking oral contraceptives. Mortality attributable to these increased risks can be estimated at between 1 and 4 deaths

per million women per year. This risk is greatest in the early years of oral contraceptive use in women who have not previously been exposed to high estrogen levels, indicating that individual susceptibility is important.

Continuing epidemiological investigation of MI in oral contraceptive users has raised the possibility that, with sufficient minimisation of accompanying risk factors (for example, cigarette smoking and elevated BP), the increased overall risks of MI with oral contraceptive use seen in the majority of studies to date appears to have been reduced to an undetectable level. This may also be the case with thrombotic and haemorrhagic stroke.

Occlusive vascular disease in oral contraceptive users is clearly a rare event with few deaths. One conclusion to be drawn from our review is that the rare cases of occlusive vascular disease that may be attributed to the oral contraceptive are atypical. Attempts to understand any of the several manifestations of vascular occlusion in these cases by analogy with similar conditions seen in individuals not taking oral contraceptives should be viewed with caution. Such attempts will have worked against resolution of the underlying causes of vascular occlusion in oral contraceptive users by diverting attention from the characteristics of the cases themselves to only those features of the cases characteristic of vascular disease in general. With regard to VTE, minimal attention has been paid to the proliferative vascular lesions, which are not a characteristic of VTE in general, but which have been described consistently in sporadically published reports of histopathology in oral contraceptive users. With regard to MI, evidence that in oral contraceptive users this is a manifestation of the condition of MI with normal angiography has been used to downplay the potential importance of oral contraceptive induced changes in endothelial function. These factors have been viewed solely in terms of their potential roles in the end-stage condition, atherosclerosis. Failure to recognise that changes in these factors could modify the consequences of existing arterial damage, and that such damage is, for the most part, angiographically invisible, has resulted in protracted confusion over the meaning of reports of increased risks of MI in oral contraceptive users.

In addition to providing a reference summary of research into vascular disease in oral contraceptive users, this work was undertaken to explore whether a unified consideration of the available evidence could provide any new insights into possible mechanisms, or provide direction for future investigations. This review therefore concludes with interpretations of the evidence with regard to VTE and arterial disease. These interpretations are speculative, the only reasonable certainty in this field of investigation being that the combined oral contraceptive is associated with an increased risk of VTE and this risk is somehow mainly associated with the estrogen component. The advantage of making such speculations, however, is that they can provide a focus for future investigations and a framework for the interpretation of new findings, which may confirm the following interpretations or require them to be modified.

Two principal factors have emerged with regard to venous thrombosis in oral contraceptive users. One is an estrogen-induced increase in the activity of enzyme systems responsible for breakdown of the vascular elastic matrix. This results in loss of vascular tone and venous stasis, and in increased endothelial permeability. Access of growth promoting substances to the subendothelial space then causes intimal and endothelial hyperplasia, disruption of the endothelial surface, and further disruption to the normal flow characteristics of the vein. Venous thrombosis then occurs in these areas of venous stasis and disrupted vascular structure. The second contributing factor, which works in concert with these changes is a potentially prothrombotic change in the balance of activities of the haemostatic system. This might itself be a consequence of endothelial disruption. Changes in activity markers indicate that oral contraceptive use is associated with a tonic low grade activation of both coagulation and fibrinolysis. This combined effect results from activation of the lumenal membranes, expression of membrane bound cofactors, and a

local shift of both coagulation and fibrinolysis to a higher level of activity. Thrombosis may then be considered as a consequence of insufficient anticoagulant or fibrinolytic capacity.

The principal candidate for the inadequate anticoagulant activity in oral contraceptive users is an acquired resistance to APC. There is: (i) decreased sensitivity to APC in oral contraceptive users; (ii) significantly reduced APC sensitivity in oral contraceptive users who have experienced a thrombotic event compared with those who have not; and (iii) in factor V Leiden mutation, we have a genetic model with which APC resistance has been unequivocally linked with increased risk of venous thrombosis. However, the meaning that can be ascribed to variation in acquired APC sensitivity in oral contraceptive users is unclear, since measurements made using an intrinsic pathway-based assay do not correlate with measurement made using the extrinsic pathway assay. In contrast to measurements using the intrinsic pathway, measurements made using the extrinsic pathway have not been consistently linked with increased risk of VTE. The influences of oral contraceptive-induced changes in factors of the intrinsic pathway (e.g. FVIII) and extrinsic pathway (e.g. FVII or tissue factor inhibitor) on measures of APC sensitivity need to be explored in greater depths.

The possibility has been raised that oral contraceptive progestogens such as levonorgestrel and norethisterone might oppose estrogen-induced increases in risk of VTE to a greater extent than desogestrel, gestodene and, historically, megestrol and chlormadinone. Current epidemiological information does not exclude such an effect, but makes clear that if it is indeed operating it is barely within the resolving power of existing studies, given the biases that may have been operating. Future studies might usefully pay increased attention to the influences of patterns of previous oral contraceptive usage on risks of VTE, short term risks in first-time ever users, and reasons for choice of one particular formulation relative to others.

At the time of writing, there is no agreed mechanism for the increased risk of VTE in oral contraceptive users despite at least 30 years of research into this problem. The risks are admittedly small. However, women using oral contraceptives do not perceive them as such. Recent reports suggesting that certain oral contraceptive formulations increased risk of (principally nonfatal) VTE from 1.5 cases per 10 000 to 3.0 cases per 10 000 were sufficient to eliminate 68% of the usage of those formulations.[768] In view of the perceived importance of such risks and the importance of effective contraception to public health, investigations into the causes of VTE in oral contraceptive users should continue, but more intensively and in a more focused manner than it has been up to now. The lack of information about direct vascular effects of contraceptive steroids is clearly a major gap in our knowledge. Moreover, uncertainty remains over the clinical significance of the manifold haemostatic factor measurements that are available. Resolution of these issues may be assisted by the greater emphasis that is developing on more inclusive measures of activity. The activities of antithrombotic mechanisms are clearly important in this respect. However, the example of *in vitro* evaluation of the sensitivity of thrombin or fibrin generation to APC in blood from oral contraceptive users indicates some of the uncertainties that may develop in the interpretation of such measures. Other activity measures relating to, for example, thrombin generation (prothrombin F_{1+2}) and thrombus formation (D-dimer) may yet prove informative and may assist in localising thrombotic risk to particular individuals. Until now, virtually all the available epidemiological data has been concerned with population effects and there remains very little information on the risks that an individual woman using oral contraceptives might be exposed to.[834] Beyond these considerations, it should be borne in mind that the weight of evidence implicates the estrogen component as the principal culprit in VTE. Orally administered ethinylestradiol has remained in use as almost the sole oral contraceptive estrogen for thirty years. If an alternative were found which carried no attendant risk of VTE,

the many outstanding issues outlined here would be rendered irrelevant.

With regard to mechanisms of MI (and quite possibly ischaemic stroke) in oral contraceptive users, there are 3 critical observations: (i) the characteristic features of MI with normal coronary angiography, (ii) the unequivocal synergy, seen primarily in earlier studies, between such risk factors as cigarette smoking and hypertension and oral contraceptive use in increasing risks of MI, and (iii) the minimisation (and possibly complete elimination) of any increased risk with minimisation of associated risk factors for MI (e.g. cigarette smoking, hypertension). These 3 core observations support the following interpretation of the evidence with regard to MI in oral contraceptive users.

There is a continuous cycle of endothelial damage and repair in the arterial system. In the presence of classic risk factors for atherosclerosis such as cigarette smoking and hypertension, this cycle is accelerated and phases of endothelial damage become more prolonged. Whether this cycle continues with no clinical consequences, whether it progresses over time to form classic atherosclerotic lesions, or whether thrombotic occlusion intervenes, depends on the balance of endothelial damage and repair, and the prothrombotic milieu in which this damage is taking place. Oral contraceptive use favours thrombotic occlusion. The synergy previously reported between, for example, heavy cigarette smoking and oral contraceptive use in risk of MI may then be understood as an intervention by the prothrombotic effects of the oral contraceptive in a cycle of endothelial damage and repair that has already been weighted in favour of endothelial damage. There appear to be thresholds at work in this synergy since, without the additional prothrombotic effects of the oral contraceptive, no thrombus forms and the tendency is instead for the smoking-induced endothelial damage to progress to atherosclerosis. Moreover, it is possible that the prothrombotic effects of the oral contraceptive have to exceed a certain threshold since, in the most recent studies no effect of oral contraceptives containing less than 0.05mg estrogen on MI risk was seen, even among heavy smokers. This contrasts with the marked synergy consistently seen in studies in which use of formulations containing estrogen 0.05mg or more predominated. There also appears to be a threshold with regard to the degree of endothelial damage sustained, since there have been consistent reports of no additional risk of MI associated with oral contraceptive use among women smoking less than 10 cigarettes per day.

Inconsistencies between studies of risks of MI in oral contraceptive users may then be understood in terms of whether the prothrombotic threshold or the arterial damage threshold was exceeded in the groups under consideration. Both thresholds need to be exceeded if a significant effect of oral contraceptives on risk of MI is to emerge. Factors not related to oral contraceptives that could determine whether the endothelial damage threshold was exceeded include increasing age, cigarette smoking and elevated BP. It should be noted that in the absence of BP checking there may, in some groups, have been individuals with undetected high BP. Therefore, there may have been appreciable endothelial damage present in groups that were apparently free of arterial disease risk factors, thus providing a further basis for inconsistencies.

Factors related to oral contraceptive use that might affect whether the endothelial damage threshold was exceeded include direct effects of the estrogen on vascular structure, increases in insulin, BP or homocysteine associated with oral contraceptive use, or, either favouring or opposing endothelial damage, variation in HDL levels associated with oral contraceptive use. Factors related to oral contraceptive use that might affect whether the prothrombotic threshold was exceeded include changes in platelet activity and adhesion, and estrogen-dependent prothrombotic changes in haemostatic activities. It will be appreciated that covariation in such factors will not have been consistently accounted for in the studies of MI in oral contraceptive users that have been published over the past 30 years. The uncertainties that have continued in this area are therefore not surprising.

The possibility has been raised that oral contraceptive formulations containing certain progestogens (e.g. norethisterone at high doses or levonorgestrel) might be associated with greater risks of arterial disease than others (e.g. norethisterone at low doses, desogestrel or gestodene). The tendency towards higher BP and insulin levels and lower HDL cholesterol levels seen in users of the former formulations would accord with this, and current epidemiological information does not exclude such an effect. However, as with VTE, if such an effect is indeed operating it is again barely within the resolving power of existing studies, given not only the biases that may be operating but also the relatively small number of cases available for study. These issues may, in any case, not have been appropriately studied. If there are thresholds for endothelial damage and prothrombotic influences in risks of MI associated with oral contraceptive use, and study groups fall below these threshold, then no differentials will be seen between different formulations because there will be no detectable increase in risk with any type of formulation. Such differentials can only be usefully examined in groups that are clearly already at risk, such as heavy smokers or those with hypertension. Women with such risk profiles are, of course, discouraged from taking the combined oral contraceptive but many, for whatever reason, do choose this form of contraception and such risks as they may be exposed to should be better understood. A major obstacle in this will be the numbers of cases available for study. Future studies of MI in oral contraceptive users might endeavour to adopt a uniform protocol so as to enable datasets to be combined, thus, increasing the numbers available for analysis.

Finally, the combined oral contraceptive is the most effective reversible contraceptive yet devised. Periodic alarms over its safety, whether or not justified in their intensity, have meant a continuing undercurrent of anxiety for women using this form of contraception. As evidenced by its continuing use by hundreds of millions of women over 40 years, these anxieties weigh to only a limited degree in contrast to the benefits of using 'the pill'. Oral contraception is, therefore, likely to remain a reality for some time to come. It is to be hoped that the present review will contribute not only to a more accurate appraisal of such risks as may accompany this, but also to continuing improvements in the safety of oral contraceptive formulations and their use.

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Dr Winkler has, over the past 5 years, been principal investigator in several multicentre trials of the haemostatic effects of oral contraceptive, funded by NV Organon and Wyeth-Ayerst, and has received occasional payments for lecturing on the haemostatic effects of sex steroids from manufacturers of oral contraceptives.

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Dr Crook has a consultancy arrangement with and receives research funding from NV Organon in connection with the metabolic effects of steroid hormones. Over the past 5 years, he has received honoraria and hospitality from various manufacturers of steroid hormones for lecturing on the subject of steroid hormones and cardiovascular risk, as well as *ad hoc* payments for advice given in connection with legal actions and regulatory authority hearings concerning the subject of steroid hormones and cardiovascular risk.

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Authors' note:

Reviews on a scale such as this are generally in edited form, with distinct aspects of the subject being covered in separate chapters, each with its own author or panel of authors. In the present work, we have attempted a broadly synthetic approach in which different aspects of the issues are discussed in the context of a single, jointly-authored,

review. Nevertheless, a breakdown of each author's principal input is given here so that questions or comments on specific aspects of the review can be directed to the appropriate individual. Section 2 on the epidemiology of occlusive vascular disease in oral contraceptive users was assembled by Drs Lidegaard and Godsland, section 4.9 on the haemostatic system by Dr Winkler, and section 4.3 on lipid and lipoprotein metabolism by Dr Crook, who also contributed substantially to section 5.6 on progestogen effects on myocardial infarction risk and undertook detailed editing. Dr Godsland was responsible for all other sections.

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