Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 260–266
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Do novel risk factors differ between men and women aged 18 to 39 years with a high risk of coronary heart disease?

Serena Tonstad[a,*], Hege Thorsrud[a], Peter A. Torjesen[c], Ingebjørg Seljeflot[b]

[a]*Department of Preventive Cardiology, Center for Preventive Medicine, Ullevål University Hospital, Oslo N-0407, Norway*
[b]*Center for Clinical Research, Ullevål University Hospital, Oslo N-0407, Norway*
[c]*Hormone Laboratory, Aker University Hospital, Oslo N-0514, Norway*

Received 5 January 2006; accepted 16 October 2006

Abstract

This study aimed to clarify whether high-risk premenopausal women have less atherogenic levels of markers of endothelial dysfunction, oxidation, thrombosis and inflammation, and adipokines than high-risk men of the same age. Thus, we studied levels of these markers and their determinants in 207 men and women aged 18 to 39 years with dyslipidemia and a family history of premature coronary heart disease. Women had favorable levels of E and P selectins, tumor necrosis factor α, tissue plasminogen activator, plasminogen activator inhibitor 1, thrombomodulin, thiobarbituric acid reactive substances, and adiponectin compared with men, but had higher levels of high-sensitivity C-reactive protein and leptin (all $P < .05$) and no difference in the L-arginine/asymmetric dimethyl arginine (ADMA) ratio. This ratio was higher among nonusers of hormonal contraception than among users ($P = .02$). In multivariate analyses, levels of intercellular adhesion molecule 1 and E selectin were associated with cigarette smoking and dietary sucrose (both $P < .05$), whereas the L-arginine/ADMA ratio was paradoxically associated with smoking ($P < .05$). Of 17 novel risk markers, 11 were associated with body mass index after adjustment for age, sex, smoking, and percentage of dietary energy from sucrose (regression coefficients, 0.14-0.62; all $P < .05$). In conclusion, the findings underscore the female advantage regarding determinants of novel risk markers in young adults at risk of coronary heart disease, although some endothelial dysfunction markers (cellular adhesion molecules, L-arginine/ADMA ratio) were not more favorable in women compared with men. Lifestyle factors including body mass index, dietary sucrose, smoking, and hormones were associated with levels of the markers independent of sex with body mass index being the most prominent factor.

1. Introduction

The classic risk factors may not account for all of the increased coronary heart disease (CHD) risk of individuals with a hereditary predisposition to atherosclerosis [1]. For example, endothelial dysfunction has been demonstrated in the arteries of young adults whose only identifiable vascular risk factor was a strong family history of CHD [2]. Furthermore, a number of novel pathways for vascular disease have emerged, and laboratory parameters that putatively reflect these mechanisms have been investigated. These include markers of prothrombotic state (thrombomodulin, tissue plasminogen activator antigen [tPAag], plasminogen activator inhibitor 1 [PAI-1], and von Willebrand factor), low-grade chronic inflammation (C-reactive protein [CRP] and proinflammatory cytokines), and measures of endothelial dysfunction or injury (soluble endothelial cell adhesion molecules). In addition, there is a preponderance of basic and clinical evidence that supports the role of oxidative processes in atherogenesis. Elevated levels of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase, have been considered to be a possible marker of endothelial dysfunction and atherosclerosis [3]. Several of these risk markers have been linked to the risk of atherosclerotic disease [4-11], and aggregate in families with CHD [12-15]. Finally, adipocyte-secreted hormones, including adiponectin and leptin, are thought to link obesity with insulin resistance and increased risk of CHD [16,17].

Premature CHD conveys a considerable increase in the risk of the disease in the relatives of index cases. This association may be mediated through hereditary or shared lifestyle influences on the causal pathways of atherosclerotic

* Corresponding author. Tel.: +47 22 11 79 39; fax: +47 22 11 99 75.
E-mail address: serena.tonstad@uus.no (S. Tonstad).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.10.006

disease. By damaging or activating the vascular endothelium, hyperlipidemia and smoking may initiate the atherosclerotic process as early as the second or third decade [18], and have been shown to predict 20-year rates of death from CHD in men aged 18 to 39 years [19]. Although premenopausal women have a substantially lower risk compared with men, men and women with genetic and lipid risk factors are a prime target for lifestyle interventions aimed at reducing the risk of CHD [20]. In the current study, we studied a wide range of novel risk factors in subjects aged 18 to 39 years with a high risk of CHD due to dyslipidemia and a family history of premature CHD or hyperlipidemia. We firstly asked whether some or all of these factors were more favorable in women than in men and secondly sought to understand the potential of lifestyle change to modify these factors by studying their determinants. The determinants of novel risk factors that we studied included age, diet, smoking, and body mass index (BMI).

2. Subjects and methods

First-degree relatives (siblings or adult offsprings) aged 18 to 39 years of patients admitted to the cardiology ward at the Ullevål University Hospital, Oslo, Norway, with premature CHD were screened for dyslipidemia. The screening program was approved by the regional ethics committee and has been described previously [21,22]. Other subjects were referred by their primary physician or had participated in other cardiovascular screening programs. Subjects were eligible for the current study if they had a fasting total cholesterol level of 4.5 mmol/L or higher with 1 or more of the following lipid abnormalities: a level of triglycerides greater than 1.5 mmol/L, high-density lipoprotein cholesterol (HDL-C) level of less than 1.0 mmol/L for men or less than 1.2 mmol/L for women, or lipoprotein (a) level above the 75th percentile; and a family history of premature CHD defined as having at least 1 first- or second-degree relative with premature CHD (men <60 years or women <65 years) and/or a documented family history of hyperlipidemia. In all, 207 men and women took part in the study, of whom 197 had relatives with premature CHD with or without hyperlipidemia, and 10 had only family members with hyperlipidemia. Individuals with a history of any cardiovascular disease, DNA-confirmed familial hypercholesterolemia, diabetes, uncontrolled blood pressure level of 160/100 mm Hg or higher on 2 or more measurements, or pharmacologically treated hyperlipidemia were excluded. Six subjects were taking antihypertensive medications. Of the 32 female subjects, 10 were taking hormonal contraception.

At the initial visit, the study physician obtained the medical history, recorded all medications including hormonal contraception for women, and performed a physical examination. Blood pressure and pulse rate were measured after the subject had rested quietly for 5 minutes. Measurements of height and weight (using a digital scale) were performed, and BMI (in kg/m^2) was calculated. Waist circumference was measured midway between the lower rib margin and the iliac crest at the end of a normal expiration. Hip girth was measured at the maximum circumference of the buttocks. Use of tobacco (number of cigarettes smoked per day and/or amount of snuff used per week) was recorded, and cigarette smoking or nonsmoking was verified with a microcarbon monoxide meter (Micro Medical, Rochester, Kent, UK).

Subjects completed a previously validated food frequency questionnaire [23]. The questionnaire surveyed the respondents' consumption of 190 food items that were selected based on Norwegian dietary patterns obtained in previous dietary studies. The subjects indicated the number of portions consumed and their frequency of consumption of the food items. The dietitian checked the questionnaire to correct or clarify missing questions and double marks and to specify portion sizes, as needed. The questionnaire was read optically, and we computed the daily intakes of nutrients and foods using a food database and a software system developed at the Section for Dietary Research at the University of Oslo [23]. Physical activity was assessed by a questionnaire regarding the frequency of walking, hiking, cycling, gymnastics, swimming, dancing, and other activities [24].

2.1. Laboratory measurements

Subjects fasted and had not smoked cigarettes or ingested vitamins during the previous 12 hours. Blood samples were drawn in the morning between 8:00 and 10:00 AM.Citrated and EDTA blood samples were collected and stored on ice until platelet-poor plasma was obtained within 30 minutes by centrifugation at 2000*g* for 20 minutes. Serum was prepared within 1 hour. All samples were kept frozen at −70°C for batch analysis of the variables except for serum lipoproteins and glucose, which were analyzed by conventional enzymatic methods. Vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), E selectin, P selectin (measured in citrated plasma), tumor necrosis factor α, and interleukin 6 were analyzed with commercial enzyme-linked immunosorbent assay (ELISA) methods (R&D System, Abingdon, Oxon, UK), and high-sensitivity CRP was determined by immunoturbidimetric methods (Cobas Integra, F. Hoffmann-La Roche, Basel, Switzerland).

Determinations of von Willebrand factor, thrombomodulin, and tPAag were done in citrated plasma using ELISA methods (Asserachrom Stago Diagnostica, Asnieres, France, and TintElize tPA, Biopool, Umeå, Sweden, respectively) as was PAI-1 activity (Spectrolyse PL, Biopool). Serum thiobarbituric acid reactive substances (TBARS) were analyzed by a colorimetric method previously described with minor modifications [25]. Oxidized low-density lipoprotein cholesterol (LDL-C) level was determined in EDTA plasma using an ELISA method (Mercodia, Uppsala, Sweden). The interassay coefficients of variation (CVs) were 5.2% for VCAM-1, 4.8% for ICAM-1, 5.3% for E selectin, 7.2% for P selectin, 8.5% for tumor necrosis factor α, 10.7% for interleukin 6, less than

5% for CRP, 8.0% for von Willebrand factor, 7.6% for thrombomodulin, 3.5% for tPAag, 4.4% for PAI-1, 12.7% for TBARS, and 7.7% for oxidized LDL-C. L-Arginine and ADMA levels were measured in EDTA plasma by high-performance liquid chromatography and precolumn derivatization with *o*-phthaldialdehyde (Sigma Chemicals, St Louis, MO) as previously described in detail [26] with a CV of less than 5%. The serum levels of adiponectin and leptin were measured with radioimmunoassay kits from Linco Research (St Charles, MO) with CVs of 10% for both variables.

2.2. Statistical analysis

Baseline characteristics of men and women as well as those women using or not using hormonal contraception were compared using a χ^2 test for categorical variables and 2-tailed unpaired *t* tests for continuous variables. Variables that were markedly skewed including triglycerides, VCAM-1, ICAM-1, thrombomodulin, CRP, interleukin 6, tumor necrosis factor α, and leptin were log transformed to allow for parametric analysis. Multiple logistic regression was used to estimate the association between the use or nonuse of hormonal contraception and the levels of novel risk factors with adjustment for age and BMI.

Dietary and lifestyle variables were tested for their relation to levels of risk markers based on previous data [27] and biological plausibility. These were physical activity, total fat, saturated fat, sucrose, and alcohol (all in percentage of daily energy), fiber (g/4.184 kJ of dietary energy), fruit and vegetables (g/4.184 kJ of dietary energy), and vitamin C (g/d). In simple regression analyses, only sucrose and fiber were related to novel marker levels. These 2 variables were highly correlated ($R = 0.4$, $P < .0001$), and only sucrose was statistically significantly related to novel markers in multivariate models. Stepwise linear regression models were used to determine the independent association of age, sex, BMI, cigarettes smoked per day, mean blood pressure, and dietary sucrose with novel risk factors. Mean blood pressure was only weakly related to a number of the novel risk markers and was not included in the final models. The same analyses were performed for levels of total cholesterol and triglycerides, as a comparison with novel risk factors. Four women and 7 men did not complete the dietary data, and mean values were substituted. The results did not differ when these subjects were excluded (data not shown). A 2-tailed *P* value of .05 or less was considered statistically significant. Statistical analyses were performed using Statview 10 software (Abacus concepts, Berkeley, CA).

3. Results

The eligibility criteria required subjects to have an elevated cholesterol level; thus, women and men had similar total cholesterol levels, but as expected, women had lower triglycerides and higher HDL-C levels (Table 1). More women than men smoked (Table 1). Dietary intake of men and women differed only in regard to total energy, alcohol, and fiber, but not in regard to fatty acid, protein, and carbohydrate composition (Table 1). Almost all (94.6%) of the participants performed 1 or more of the queried physical activities at least once a week.

Women had lower levels of selectins, tumor necrosis factor α, tPAag, PAI-1, thrombomodulin, TBARS, and L-arginine than men. Levels of CRP, leptin, and adiponectin were higher in women (Table 2). These differences remained statistically significant in the multivariate analysis (Table 3). The levels of cell adhesion molecules, interleukin 6, von Willebrand factor, oxidized LDL-C, ADMA and the L-arginine/ADMA ratio did not differ between the sexes (Tables 2 and 3). Women who were taking hormonal contraception (47% of all) had lower levels of interleukin 6 and PAI-1; higher levels of adiponectin and a tendency to lower tPAag and leptin levels; and higher ADMA levels than women not taking hormonal contraception (Table 2). After adjustment for age and BMI, only the difference in levels of PAI-1 and the L-arginine/ADMA ratio between users and nonusers of hormonal contraception remained statistically significant ($P = .03$ and $P = .02$, respectively).

In multiple regression analyses, E selectin, tumor necrosis factor α, interleukin 6, CRP, tPAag, PAI-1, von Willebrand factor, oxidized LDL-C, ADMA, L-arginine/ADMA, adiponectin, and leptin levels were related to BMI after adjustment for age, sex, cigarettes smoked per day, and

Table 1
Participant characteristics

Variable	Men (n = 142)	Women (n = 65)	*P*
Age, y (SD)	31 (5)	30 (5)	NS
BMI, kg/m^2 (SD)	27.7 (3.8)	26.9 (5.7)	NS
Waist circumference, cm (SD)	98 (10)	85 (13)	<.0001
Hip circumference, cm (SD)	104 (7)	103 (10)	NS
Daily cigarette smoker	34%	55%	.003
Systolic BP, mm Hg (SD)	121 (12)	114 (12)	.0005
Diastolic BP, mm Hg (SD)	78 (10)	73 (11)	.002
Heart rate, beats/min (SD)	68 (12)	73 (9)	.002
Total cholesterol, mmol/L (SD)	6.2 (1.0)	6.1 (1.0)	NS
HDL-C, mmol/L (SD)	1.1 (0.22)	1.4 (0.4)	<.0001
Triglycerides, mmol/L (SD)	2.2 (1.7)	1.7 (1.2)	.03
Apolipoprotein B, g/L (SD)	1.3 (0.2)	1.3 (0.2)	NS
Dietary intake			
Energy (kJ) (SD)	11.07 (3.95)	8.84 (2.56)	.0001
Total fat, % of energy (SD)	32.6 (5.3)	33.0 (5.0)	NS
Saturated fat, % (SD)	12.5 (5.3)	12.8 (2.2)	NS
Monounsaturated fat, % (SD)	4.8 (0.8)	4.8 (0.7)	NS
Polyunsaturated fat, % (SD)	3.1 (1.0)	3.1 (0.8)	NS
Protein, % (SD)	15.0 (2.3)	14.8 (2.6)	NS
Carbohydrate, % (SD)	48.8 (5.8)	50.2 (6.2)	NS
Sucrose, % (SD)	10.3 (5.7)	12.2 (9.0)	NS
Alcohol, % (SD)	3.1 (2.8)	1.5 (1.8)	<.0001
Cholesterol, mg/4.184 kJ (SD)	116 (31)	116 (30)	NS
Fruit/vegetables, g/4.184 kJ (SD)	173 (112)	181 (74)	NS
Fiber, g/4.184 KJ (SD)	8.9 (2.6)	9.8 (2.8)	.04

NS indicates not significant.

Table 2
Levels of novel risk factors

	Men (n = 142)	Women (n = 65)	P	Nonusers of HC (n = 34)	Users of HC (n = 31)	P
E selectin (ng/mL)	58.2 (23.6)	41.0 (22.4)	<.0001	42.9 (23.5)	39.0 (21.2)	NS
P selectin (ng/mL)	39.8 (12.2)	31.5 (9.9)	<.0001	32.6 (10.1)	30.3 (9.7)	NS
VCAM-1 (ng/mL)	369 (315, 457)	351 (298, 428)	NS	352 (299, 428)	345 (294, 423)	NS
ICAM-1 (ng/mL)	252 (211, 287)	264 (221, 301)	NS	266 (222, 314)	251 (221, 288)	NS
Tumor necrosis factor α (pg/mL)	2.2 (1.3, 3.5)	1.9 (1.0, 2.7)	.04	2.1 (1.3, 2.7)	1.9 (0.9, 2.9)	NS
Interleukin 6 (pg/mL)	1.6 (1.2, 2.4)	1.9 (1.3, 3.1)	NS	2.3 (1.5, 3.3)	1.7 (1.1, 4.7)	.04
High-sensitivity CRP (mg/L)	1.40 (0.78, 2.65)	2.35 (0.96, 5.73)	.005	2.07 (0.74, 5.66)	2.74 (1.04, 5.89)	NS
tPAag (ng/mL)	8.3 (2.7)	5.6 (3.0)	<.0001	6.3 (3.0)	4.9 (2.7)	.06
PAI-1 (U/L)	15.1 (8.7)	11.2 (8.0)	.003	14.1 (9.3)	8.2 (4.7)	.003
Thrombomodulin (ng/mL)	40.5 (34.3, 48.5)	36.2 (28.8, 44.1)	.02	39.6 (30.0, 45.5)	35.5 (27.9, 41.8)	NS
von Willebrand factor (%)	133 (38)	136 (42)	NS	132 (43)	140 (43)	NS
Oxidized LDL-C (U/L)	63.1 (18.5)	65.1 (20.1)	NS	69.5 (19.4)	60.3 (20.1)	NS
TBARS (μmol/L)	1.38 (0.51)	1.15 (0.39)	.001	1.21 (0.33)	1.08 (0.44)	NS
ADMA (μmol/L)	1.54 (0.58)	1.39 (0.63)	NS	1.25 (0.62)	1.55 (0.62)	.06
L-Arginine (μmol/L)	84 (13)	76 (15)	.0002	78 (13)	73 (17)	NS
L-Arginine/ADMA	62 (27)	68 (38)	NS	81 (45)	53 (21)	.004
Adiponectin (ng/mL)	6993 (2626)	9992 (3770)	<.0001	8867 (4010)	11226 (3101)	.01
Leptin (pmol/L)	517 (338)	1316 (827)	<.0001	1500 (982)	1115 (563)	.06

Values are expressed as mean (SD) or median (interquartile range). Values of some variables were missing for 1 to 6 subjects. HC indicates hormonal contraception; hs-CRP, high-sensitivity CRP.

dietary sucrose (Table 3). Levels of E selectin and ICAM-1 were independently related to the number of cigarettes smoked per day; furthermore, tPAag level was related to dietary sucrose. Sex, BMI, smoking, and dietary sucrose contributed significantly to the variance in E selectin level; in contrast, none of the variables were associated with the level of VCAM-1 (Table 3). The number of cigarettes smoked per day was positively associated with L-arginine/ADMA levels and inversely with leptin levels (Table 3). The variance in leptin level was otherwise accounted for by BMI and sex. Altogether, the amount of variance that could be explained for each novel factor varied from none for VCAM-1 to 72.6% for leptin.

To further clarify the relationship between novel risk factors and sex, standardized linear regression coefficients between novel risk factors and HDL-C level were calculated (Table 4). Tumor necrosis factor α, tPAag, PAI-1, thrombomodulin, ADMA, and adiponectin remained associated with HDL-C after adjustment for sex in addition to the adjustment for age, BMI, and smoking. On the other

Table 3
Standardized multivariate linear regression coefficients adjusted for age, sex, BMI, cigarettes smoked per day, and percentage of dietary energy from sucrose

	Age	Sex[a]	BMI	Cigarettes per day	Dietary sucrose	R^2 (%)	95% CI
Total cholesterol	0.18*	–	0.20**	–	–	8.4	2.5-16.8
Triglycerides	0.17*	0.18*	0.36***	–	0.13*	22.6	12.3-27.1
Novel factors							
E selectin	–	0.36***	0.33***	0.19**	0.21**	28.9	19.0-39.7
P selectin	–	0.33***	–	–	–	5.6	1.1-13.4
VCAM-1	–	–	–	–	–	–	
ICAM-1	–	–	–	0.35***	0.13*	14.9	7.1-25.0
Tumor necrosis factor α		0.15*	0.19**	–	–	5.6	1.1-13.4
Interleukin 6	–	–	0.25**	–	–	8.9	2.9-17.7
Hs-CRP	–	−0.26***	0.43***	–	–	23.9	14.2-34.6
tPAag	0.15*	0.39***	0.41***	–	0.13*	38.8	27.9-48.7
PAI-1	–	0.17*	0.53***	–	–	33.1	23.0-44.8
Thrombomodulin	–	0.17*	–	–	–	2.9	0.1-9.1
von Willebrand factor	–	–	0.16*	–	–	2.6	0.05-8.5
Oxidized LDL	0.23**	–	0.14*	–	–	6.7	1.6-14.7
TBARS	–	0.22**	–	–	–	5.0	0.7-11.2
ADMA	–	–	0.22**	–	–	4.1	0.4-10.8
L-arginine/ADMA	0.17*	–	−0.26***	0.17*	–	11.0	4.1-20.1
Adiponectin	−0.16*	−0.39***	−0.24**	–	–	28.5	17.9-38.7
Leptin	–	−0.64***	0.62***	−0.08*	–	72.6	65.1-78.1

CI indicates confidence interval.
[a] Female = 0, male = 1.
* $P < .05$.
** $P < .01$.
*** $P < .0001$.

Table 4
Association of HDL-C with the novel risk markers

	Standardized regression coefficient			
	Age, BMI, and smoking		Age, BMI, smoking, and sex	
	Adjusted	*P*	Adjusted	*P*
E selectin	−0.20	.004	–	
P selectin	−0.23	.003	–	
VCAM-1	–		–	
ICAM-1	–		–	
Tumor necrosis factor α	−0.37	<.0001	−0.36	<.0001
Interleukin 6	–		–	
Hs-CRP	–		–	
tPAag	−0.32	<.0001	−0.18	.006
PAI-1	−0.19	.003	−0.14	.04
Thrombomodulin	−0.26	.0006	−0.22	.007
von Willebrand factor	–		–	
Oxidized LDL-C	–		−.16	.045
TBARS	−0.17	.02	–	
ADMA	−0.18	.01	−0.17	.04
L-Arginine/ADMA	–		–	
Adiponectin	0.42	<.0001	0.30	<.0001
Leptin	0.26	<.0001	–	

hand, the relation of selectins, TBARS, and leptin to HDL-C was no longer statistically significant after adjustment for sex (Table 4).

4. Discussion

In this cross-sectional study, we explored the hypothesis that these novel risk markers may play a role in the markedly low incidence of CHD in premenopausal women and examined the association of lifestyle with the novel markers. Our main findings were that women with an elevated risk of CHD had mostly lower and more favorable levels of a number of novel risk markers than men, suggesting that these markers could contribute to their reduced risk of CHD. Levels of CRP and leptin (increased risk) as well as of adiponectin (decreased risk), which are all closely associated with adipose tissue, were higher in women. Most of the novel risk markers were related to BMI. Endothelial adhesion molecule levels (specifically E selectin and ICAM-1) and tPAag were associated with the percentage of dietary energy from sucrose. E selectin and ICAM-1 levels were associated with smoking. Thus, lifestyle factors were the main determinants of novel risk markers in addition to sex.

4.1. Effect of sex

Premenopausal women have more favorable lipids and blood pressure levels than men, and our study confirms that they also seem to have more favorable levels of several novel markers, also after adjustment for BMI, smoking, and dietary differences. Previous studies comparing men and women have largely been conducted in older or diseased individuals [11,26,28,29], although sex differences have been better studied in regard to the more established hemostatic parameters [30]. The prevalence of smoking among the women in our study was surprisingly high, most likely because of their being selected from high-risk families and the prerequisite of dyslipidemia for participation in the study. Despite these higher rates of smoking, and after adjustment for smoking, women had lower levels of selectins, prothrombotic markers, and tumor necrosis factor α, and higher levels of adiponectin. Restriction of the analyses to smokers did not change this conclusion (data not shown).

Because a major mechanism of the decreased risk of CHD in women is thought to be their higher levels of HDL-C, we examined the relation of the various novel risk factors to the level of HDL-C both with and without adjustment for sex (Table 4). With the exception of TBARS, novel factors that were favorable in women (Table 2) were associated with level of HDL-C, suggesting that the novel factors that are associated with elevated levels of HDL-C may further reduce women's risk of CHD. In the case of tumor necrosis factor α, prothrombotic markers, ADMA, and adiponectin, the association with HDL-C remained after adjustment for sex, plausibly reflecting the underlying biological pathways and a possible mechanistic basis for the findings. For example, hypoadiponectinemia is associated with decreased plasma lipoprotein lipase and thereby low HDL-C levels [31], whereas tumor necrosis factor α plays a role in triglyceride metabolism [32].

Asymmetric dimethyl arginine is a risk factor for CHD in men; however, to our knowledge, its relation to hard end points in women has not been studied, with the exception of a study done in patients with end-stage renal disease [3]. The level of L-arginine was higher in men than in women in this study as was observed previously [26], although the L-arginine/ADMA ratio, possibly a more physiological indicator of endothelial function [26], did not differ between the sexes. Women who used hormonal contraception had a less favorable L-arginine/ADMA ratio than women who were not taking hormonal contraception, also after adjustment for differences in age and BMI between the 2 groups. In line with this, estrogen therapy does not seem to improve the L-arginine/ADMA ratio [33].

Most of the studies showing an association between the novel markers and the risk of atherosclerotic disease have been performed in men [5,7,8,16,17], with the exception of the relationship of CRP with cardiovascular disease, which is quite well documented in women [10,34]. One of the perplexities in understanding the role of inflammation in CHD is the consistent finding of higher CRP levels in women than in men despite the clearly reduced risk of CHD in premenopausal women even when markers of insulin resistance, including insulin and C-peptide levels, are similar between the sexes, as in this study (data not shown). In line with this is the observation that exogenous estrogen increases levels of CRP [35] and may increase the risk of CHD [36]. Likewise, although a role of leptin as a possible cause of vascular disease has been suggested [17], women

have higher leptin levels than men due to differential body fat distribution, effects of sex steroids, or unknown factors [37].

4.2. Effect of BMI

The findings underscore the pivotal role played by adipose tissue in determining levels of many of the novel risk factors. The close relation between several of the novel risk markers and BMI is compatible with the adipose tissue origin of some of these factors, including tumor necrosis factor α, interleukin 6, leptin, and adiponectin, as previously established [35]. Interleukin 6, a major proinflammatory cytokine, is produced in adipocytes, as well as a variety of other tissues. Likewise, the association of adhesion molecules with BMI reflects increased expression induced by proinflammatory cytokines. The association between BMI and novel markers (other than leptin) was most marked in regard to levels of tPAag and PAI-1. The significant relationship between ADMA and BMI is in line with our previous findings in a population of older high-risk men [38], suggesting a relationship between obesity and endothelial dysfunction. The finding of a relation between oxidized LDL-C levels and BMI was concordant with the relation between total cholesterol levels and BMI (and between LDL-C level and BMI; data not shown), and leptin-dependent low-density lipoprotein oxidation has been suggested as a novel link between obesity and atherosclerosis [39]. This link was also noted in our data set (regression coefficient, 0.16; $P = .02$).

4.3. Effect of diet and smoking

Various dietary factors may attenuate the expression of endothelial adhesion molecules, including fatty acids derived from fish and dietary antioxidants [27]. We are not aware of previous data linking dietary sucrose with the novel risk markers, as observed here. The association between some of the novel risk factors and dietary energy from sucrose was on the order of the magnitude of the association between triglycerides and dietary sucrose (weak but statistically significant). An increased dietary sucrose level may be a marker of a diet with a poor nutritional density and was negatively correlated with fruit and vegetables (regression coefficient, −0.23; $P = .001$), fiber, and vitamin C (regression coefficient, −0.19; $P = .008$); however, only sucrose was associated with the novel factors.

Several risk factors (ICAM-1, E selectin) were related to cigarette smoking, in agreement with previous studies [28,29]. We have previously reported that smoking cessation reduced levels of ICAM-1 in a subset of this cohort [22], suggesting a causal relation between smoking and ICAM-1 metabolism. In accordance with our finding of a negative relation between smoking and leptin, short-term cigarette smoking has been shown to reduce plasma leptin levels [40]. The somewhat paradoxical and not easily explainable positive relationship between smoking and L-arginine/ADMA found in these young individuals has been reported earlier in an elderly high-risk population [38].

4.4. Study limitations

Several limitations of our study warrant discussion. It is clearly not possible in a cross-sectional study to attribute causality to any of the sets of correlated variables. We did not measure psychological and socioeconomic variables that could influence novel risk factors. We did not measure body fat directly, but we used BMI as a surrogate for body fat; however, the results were similar when waist circumference was substituted for BMI (data not shown). Almost all participants reported some form of physical activity in our questionnaire. Thus, we were not able to distinguish the effect of physical activity on the novel risk markers. Some selection bias may be present because women are more likely to participate in the screening of relatives than men [21].

5. Conclusions

Premenopausal women have lower rates of coronary disease than similar-aged men, even in the setting of hyperlipidemia and positive family history of premature CHD. The findings from the current study show that high-risk women with dyslipidemia and a family history of premature CHD or hyperlipidemia have favorable levels of a variety of novel risk markers thought to play a role in the pathogenesis of atherosclerosis and acute coronary syndromes including markers of endothelial activation, prothrombosis, inflammation, and adipose tissue function. Other novel markers, including cellular adhesion molecules, L-arginine/ADMA ratio, CRP, and leptin, did not differ between high-risk young men and women. It remains unknown whether these differences in novel risk markers can account for the sex differences in the risk for clinical cardiovascular events in these subjects. It is notable that several of these markers are correlated with diet and smoking, suggesting that they may be modified by lifestyle interventions.

Acknowledgments

The work was supported in part by a grant from the Norwegian Foundation for Health and Rehabilitation.

We thank Ragnhild Kleve and Hanne S. Kleven for the excellent technical and laboratory assistance, Hilde M.A. Eid for the analyses of ADMA and arginine, Tine Sundfør for the assistance with the dietary assessments, and Helene Bergstrøm, Tor Ole Klemsdal, and Axel Marco Meo for some of the medical evaluations.

References

[1] Snowden CB, McNamara PM, Garrison RJ, Feinleib M, Kannel WB, Epstein FH. Predicting coronary heart disease in siblings—a multivariate assessment. Am J Epidemiol 1982;115:217-22.

[2] Clarkson P, Celermajer DS, Powe AJ, Donald AE, Henry RM, Deanfield JE. Endothelium-dependent dilatation is impaired in young

adults with a family history of premature coronary disease. Circulation 1997;96:3378-83.
[3] Vallance P. Importance of asymmetrical dimethylarginine in cardiovascular risk. Lancet 2001;358:2096-7.
[4] Ridker P, Vaughan D, Stampfer M, Manson J, Hennekens C. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet 1993;341:1165-8.
[5] Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma levels of soluble intercellular adhesion molecule–1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88-92.
[6] Salomaa V, Matei C, Aleksic N, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. Lancet 1999;353:1729-34.
[7] Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma levels of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767-72.
[8] Malik I, Danesh J, Whincup P, et al. Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. Lancet 2001;358:971-5.
[9] Cesari M, Penninx BW, Newman AB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation 2003;108:2317-22.
[10] Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004; 351:2599-610.
[11] Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387-97.
[12] Pankow JS, Folsom AR, Province MA, et al, on behalf of the Atherosclerosis Risk in Communities investigators and Family Heart Study Research Group. Family history of coronary heart disease and hemostatic variables in middle-aged adults. Thromb Haemost 1997;77:87-93.
[13] Mills JD, Mansfield MW, Grant PJ. Tissue plasminogen activator, fibrin D-dimer, and insulin resistance in the relatives of patients with premature coronary artery disease. Arterioscler Thromb Vasc Biol 2002;22:704-9.
[14] Rohde LEP, Hennekens CH, Ridker PM. Cross-sectional study of soluble intercellular adhesion molecule–1 and cardiovascular risk factors in apparently healthy men. Arterioscler Thromb Vasc Biol 1999;19:1595-9.
[15] Margaglione M, Cappucci G, Colaizzo D, Vecchione G, Grandone E, DiMinno G. C-reactive protein in offspring is associated with the occurrence of myocardial infarction in first-degree relatives. Arterioscler Thromb Vasc Biol 2000;20:198-1203.
[16] Pichon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291: 1730-7.
[17] Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104:3052-6.
[18] Gaeta G, de Michele M, Cuomo S, et al. Arterial abnormalities in the offspring of patients with premature myocardial infarction. N Engl J Med 2000;343:840-6.
[19] Nava-Nacher EL, Colangelo L, Beam C, Grennland P. Risk factors for coronary heart disease in men 18 to 39 years of age. Ann Intern Med 2001;134:433-9.
[20] De Backer G, Ambrosioni E, Borch-Johnsen K, et al. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and other societies on cardiovascular disease prevention in clinical practice. Eur Heart J 2003;24:1601-10.
[21] Tonstad S, Westheim A. Implementation of guidelines to screen relatives of patients with premature CHD. Am J Cardiol 2002; 90:1211-4.
[22] Tonstad S, Sundfør T, Seljeflot I. Effect of lifestyle changes on atherogenic lipids and endothelial cell adhesion molecules in young adults with familial premature coronary heart disease. Am J Cardiol 2005;95:1187-91.
[23] Andersen LF, Solvoll K, Johansson LRK, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighted records, fatty acids and alpha-tocopherol in adipose tissue and serum. Am J Epidemiol 1999;150:75-87.
[24] Anderssen SA, Damm FR, Bjerkan K, Løvø A, Hustvedt BE. Registration of physical activity and energy expenditure: validity control of a questionnaire. Med Sci Sport Exerc 2000;(Suppl 32):81.
[25] Hostmark AT, Lystad E. Growth inhibition of human hepatoma cells (HepG2) by polyunsaturated fatty acids. Protection by albumin and vitamin E. Acta Physiol Scand 1992;144:83-8.
[26] Eid HM, Eritsland J, Larsen J, Arnesen H, Seljeflot I. Increased levels of asymmetric dimethylarginine in populations at risk for atherosclerotic disease. Effects of pravastatin. Atherosclerosis 2003; 166:279-84.
[27] Hinderliter AL, Caughey M. Assessing endothelial function as a risk factor for cardiovascular disease. Curr Atheroscler Rep 2003;5: 506-13.
[28] Schumacher A, Seljeflot I, Sommervoll L, Christensen B, Otterstad JE, Arnesen H. Increased levels of markers of vascular inflammation in patients with coronary heart disease. Scand J Clin Lab Invest 2002;62:59-68.
[29] Ponthieux A, Herbeth B, Droesch S, Haddy N, Lambert D, Visvikis S. Biological determinants of serum ICAM-1, E-selectin, P-selectin and L-selectin levels in healthy subjects: the Stanislas study. Atherosclerosis 2004;172:299-308.
[30] Eliasson M, Evrin P-E, Lundblad D, Asplund K, Rånby M. Influence of gender, age and sampling time on plasma fibrinolytic variables and fibrinogen. Fibrinolysis 1993;7:316-23.
[31] von Eynatten M, Schneider JG, Humpert PM, et al. Decreased plasma lipoprotein lipase in hypoadiponectinemia. Diabetes Care 2004; 27:2925-9.
[32] Jovinge S, Hamsten A, Tornvall P, et al. Evidence for a role of tumor necrosis factorα in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. Metabolism 1998; 47:113-8.
[33] Post MS, Verhoeven MO, Van der Mooren MJ, et al. Effect of hormone replacement therapy on plasma levels of the cardiovascular risk factor asymmetric dimethylarginine: a randomized, placebo-controlled 12-week study in healthy early postmenopausal women. J Clin Endocrinol Metab 2003;88:4221-6.
[34] Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.
[35] Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler Thromb Vasc Biol 2002;22:1668-73.
[36] Herrington DM, Klein KP. Randomized clinical trials of hormone replacement therapy for treatment or prevention of cardiovascular disease: a review of the findings. Atherosclerosis 2003;166:203-12.
[37] Rosenbaum M, Nicolson M, Hirsch J, et al. Effects of gender, body composition, and menopause on plasma levels of leptin. J Clin Endocrinol Metab 1996;81:3424-7.
[38] Eid HMA, Arnesen H, Hjerkinn EM, Lyberg T, Seljeflot I. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. Metabolism 2004;53:1574-9.
[39] Porreca E, Di Febbo C, Moretta V, et al. Circulating leptin is associated with oxidized LDL in postmenopausal women. Atherosclerosis 2004;175:139-43.
[40] Reseland JE, Mundal HH, Hollung K, et al. Cigarette smoking may reduce plasma leptin concentration via catecholamines. Prostaglandins Leukot Essent Fat Acids 2005;73:43-9.