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Physiological importance of ω-3/ω-6 polyunsaturated fatty acids in man. An overview of still unresolved and controversial questions

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Summary. The 'essentiality' of (ω-6) and (ω-3) fatty acids in mammals is well known. Nevertheless, some important points remain unclear concerning their implication in physiology. After a short discussion about the definition of essential fatty acids deficiency, this brief overview deals with some of these points, pointing out some of the unresolved questions.
Different subjects are approached concerning the (ω-6) and (ω-3) fatty acids metabolism: desaturases, eicosanoids, production, as well as some of their metabolic effects on cell membranes, intestinal function, glucose and lipid metabolism, haemorheology.
Key words. Essential fatty acids; deficiency; metabolism; mammals.

For the past ten years, a growing interest has been focused on the unsaturated fatty acids (FA), mainly on the polyunsaturated fatty acids (PUFA) and especially on those of the (ω-3) and (ω-6) series (ω indicates positioning of double bonds). Many data have been published, mostly concerning their effects on lipid metabolism and on prevention of coronary heart disease. It is therefore not useless to remind us that some of the PUFA are essential fatty acids (EFA).
As always, the most difficult task resides in defining words. What is the meaning of 'essential'? Some FA have been called 'essential' for two reasons: firstly, because their absence provokes an impairment of the quality of health; secondly, because the organism is unable to synthesize them, and must obtain them from the diet. In fact, the word 'essential' is sometimes used wrongly. Epidemiological data have shown that, in order to prevent coronary heart disease and atherosclerosis, it is necessary to consume a certain daily amount of PUFA. However, some PUFA are not 'essential' but, like other nutrients, may be very useful in preventing illness.
'Essential' means more than 'useful'. It means that these PUFA are required to establish the physiological conditions of normal body development and to achieve physiological functioning. It also means that these PUFA have to be present in the diet. Some aspects of 'essentiality' are still questionable, as shown by Lands[45].

Obviously, it is not within the scope of this short overview to describe deeply and exhaustively the physiological effects of (ω-3) and (ω-6) PUFA, which are still under discussion. Our aim is just to point to some of them.

EFA deficiency

Clinical and biological symptoms of (ω-6) FA deficiency have been well known since the discovery of EFA by Burr and Burr[12] and their first use in human therapy by Hansen[25, 26]. A general review of EFA deficiency in humans was written by Holman[32], and experimental deficiency was studied by Collins et al.[16] and by Wene et al.[88] using either intravenous feeding with a fat-free solution, or nasogastric drip feeding with a fat-free diet.
Less is known about deficiency of the (ω-3) FA in man. In 1982, Holman[33] observed visual and cerebral disturbances in a 6-year-old girl who had an abdominal shot wound, who was fed intravenously a formula low in (ω-3) fat and high in (ω-6) fat. The symptoms quickly disappeared after a sufficient amount of (ω-3) FA was supplied. Bjerve[4] has recently reported (ω-3) FA deficiency in 9 patients who had been on drip-feeding for 2.5 to 9 years, who received only 0.02–0.09% of their energy as (ω-3) FA. He observed 'a scaly sandy haemorrhagic dermatitis, haemorrhagic folliculitis of the scalp, growth

retardation and impaired wound healing'. Since all patients had extensive brain damage, accurate evaluation of the effect of (ω-3) FA deficiency on brain functioning was impossible. A dietary supplementation using alpha-linoleic acid at 0.2–0.3% of total energy removed all the symptoms of deficiency.

Two questions concerning these deficiencies remain unsolved. The first question is a practical one: which are the first and most sensitive clinical or biological symptoms of a beginning EFA deficiency? The second is more theoretical: does a relative EFA deficiency really exist? It is well known that the symptoms of deficiency in rats may be exacerbated by environmental factors, e.g. decreased relative humidity, restriction of water intake, increased intake of sugar or saturated fat [30, 31, 45, 73, 83], or increased intake of cholesterol or *trans*-fatty acids, an unusual amino acid content of the diet, or iron or pyridoxine deficiency [73]. It is now accepted that requirements increase under some physiological conditions like old age, pregnancy and growth, or during reproductive activity in males [74].

EFA and intestinal function

Unlike the saturated fatty acids, that are included in chylomicrons through the lymphatic pathway, most of the PUFA pass directly through the cytosol and the basolateral membrane and enter the portal venous system [81]. The structure of the intestinal brush border membranes can be modified by the supply of fats.

PUFA deficiency increases the production of eicosatrienoic acid and the triene/tetraene ratio in enterocytes. Villosities and crypts decrease in size while microvillosities increase in size. The cholesterol and phospholipid content of the border membranes in unchanged. The degree of unsaturation decreases but the unsaturation index is unchanged. Biophysical studies have shown that the lipid matrices of vesicles are more rigid than those of controls [14]. These structural changes appear to influence intestinal functions. For instance, eicosapentaenoic acid (20:5 (ω-3)) reduces the jejunal uptake of glucose [80, 82] whereas alpha-linolenic acid (18:3 (ω-3)) induces the opposite effect. The (20:5 (ω-3)) FA does not seem to modify the absorption of cholesterol, but has various effects on the absorption of medium and long chain FA [80].

In vitro absorption of cholesterol by intestinal everted sacs and brush border vesicles may be influenced [79] or may not be influenced [65] by a saturated, unsaturated or control diet. In vivo, effects obtained using vegetable oils are difficult to explain, as the low amount of phytosterols in these oils has a moderate inhibiting effect on intestinal cholesterol absorption [65].

Many questions concerning the effects of different fats on the absorption of nutrients and concerning the mechanisms of the brush border membrane adaptation to various conditions and to the fat composition of the diet remain unanswered. Neither do we know whether the experimental results obtained in rats and pigs are significant for humans and for the conventional food habits of man.

Factors influencing the delta desaturases

The activities of the desaturases, which are mainly located in the endoplasmic reticulum, are modulated by nutritional and hormonal factors:

Modulation by nutrients

A large amount of data have yielded contradictory results. These may be explained by differences in experimental protocols with regard to the quantity of nutrient intake or the measurements of desaturase activity [10, 38, 51].

The delta-9-desaturase activity is increased by the replacement of starch by simple carbohydrates or by saturated fatty acids: it is also increased by cholesterol or protein intake. Conversely, desaturase activity is decreased by linoleic and arachidonic acids, triolein, (ω-3) PUFA, ethanol, and fasting. The effect of trans-trans (18:2 (ω-6)) FA has not been elucidated; the effect of the replacement of simple carbohydrates by starch is under discussion.

The delta-6-desaturase activity is increased by protein intake and decreased by many factors, e.g. linoleic and arachidonic acids, triolein, (ω-3) PUFA, trans-trans (18:2 (ω-6)) FA, cholesterol, ethanol, zinc and fasting.

The delta-5-desaturase activity is increased by the replacement of saturated FA or triolein, by starch intake and by protein intake, and decreased by cholesterol and ethanol. Whether activity is increased by fasting or by linoleic and arachidonic acids is still under discussion.

Modulation by fatty acids

PUFA inhibit the desaturation of other fatty acids by competing at the delta-6 and delta-5-desaturase level [6, 9, 29, 37]. It has been well demonstrated that an increased intake of linoleic acid produces a decrease in the level of (ω-3) PUFA [20:5 (ω-3); 22:5 (ω-3); 22:26 (ω-3)] in the adipose tissue of rats receiving 1% of their energy as linolenic acid. Conversely, an increased intake of linolenic acid strongly decreases the content of (ω-6) PUFA in the adipose tissue. However, the mechanisms of these effects are not completely elucidated. For instance, various dietary fats have different effects on the biosynthesis of arachidonic acid but induce only slight changes of the arachidonic level in tissue lipids [75]. The influence of fats on the delta-6-desaturase system is probably of less importance than the competition of the various fatty acids for the various other fatty acid metabolic pathways. As Zevenberger and Houtsmuller say about *trans*-fatty acids, alpha-linolenic acid, oleic acid: 'Whether a low arachidonic acid biosynthesis must be regarded as an undesirable effect of these fatty acids or is of no physiological significance is still under debate.' Finally, the dietary (ω-3) FA do not change the FA composition of phospholipids of different cells and tissues in the same

way; this fact must be taken into account when one evaluates the effects of supplementation with them on the whole body[90].

Modulation by hormones

The activities of these three desaturases are also dependent on insulin; the effect of insulin on the gene transcription of delta-9-desaturase has been proved in the adipose tissue of diabetic rats[22]. The stimulating action of simple carbohydrates (glucose and fructose) is due to both insulin and carbohydrates acting on the biosynthesis of this desaturase[39,61]. Epinephrine and thyroxine also increase the delta-9-desaturase activity.

Delta-6 and delta-5-desaturase activities are stimulated by insulin but only delta-6-desaturase activity is stimulated by thyroxin. Glucagon, epinephrine and glucocorticoids decrease the delta-6-desaturase activity: glucagon and epinephrine decrease the delta-5-desaturase activity. The mechanisms of these effects are unclear[50]. Circadian variations in the activity of desaturases are also insufficiently documented.

Modulation by aging

It is generally accepted that the delta-6-desaturase activity declines with age. This has been demonstrated in the rat testis[2]. The onset of this decline varies from organ to organ: it happens earlier in the testis (3rd to 4th week)[2] than in the liver (end of 1st year)[58]. This decline can be observed in microsomes[19]. Therefore the supplementation of the diet with gamma-linolenic acid (GLA, 18:3 (ω-6)) has been proposed for elderly subjects: indeed, the intake of relatively small amounts of GLA (360 mg/day) produces an increase in dihomogamma-linolenic (20:3 (ω-6)) and in arachidonic acid blood levels[53].

Is it advisable to short-circuit the delta-6-desaturase? The cellular accumulation of aging pigment (lipofuchsin) may be due to the peroxidation of membrane fatty acids[40,78]. Peroxidation reactions, which seem to be more important in older than in younger age-groups, produce free radicals. The rate of peroxidation was found to increase with the degree of unsaturation of the FA[89]. The right balance between (ω-6) and (ω-3) FA in the diet seems to be an important factor in lipid peroxidation. Studies of the effects of various types of fats on the lipid peroxidation status of the heart in rats have shown that the ω-6/ω-3 ratio of the diet is probably of greater significance than the polyunsaturated/saturated ratio for the heart lipid peroxidation status[55]. Supplementation with GLA could constitute a potential hazard and might even accelerate the aging process. Moreover, when dietary antioxidants such as vitamin E are low in the diet, increased autooxidation and lipid peroxidation may produce an increased level of carcinogenic products.

As energy intake averages 6.69 MJ (1600 kcal) in the elderly, vitamin E intake is low. One may thus wonder whether a supplementation with GLA might not produce a short-term beneficial but a long-term detrimental effect. Furthermore, data on the (ω-6) FA status of the elderly population are lacking as no important survey of healthy people in older age-groups has touched on this problem. A recent Norwegian survey of 735 subjects aged 12–89 measured plasma level of phospholipid FA and showed that the absolute concentrations of palmitic, stearic, oleic, linoleic, dihomogammalinolenic, arachidonic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids increased from the third to the fifth decade of life, but that these levels remained fairly constant from the fifth to the ninth decade. Alpha-linoleic acid levels did not change. When the results were expressed relative to 100 g of FA, it was shown that palmitic, stearic and linoleic acids decreased from the 3rd to the 5th decade while dihomogammalinolenic and arachidonic acid remained unchanged and the long-chain (ω-3) FA increased. The availability of the (ω-3) fatty acids is very slighty modified by the aging process. It thus appears that there is still a great need for additional research and epidemiological studies before we are able to make proposals to supplement the diets of elderly people with PUFA or to short-circuit the delta-6-desaturase with GLA. The latter is contra-indicated in epilepsy and several other disorders, and we do not know about its action on psychiatric disorders that are common in the elderly, i.e. senile dementia, depression or manic-depressive disease[5,89]. We thus need additional research on the decline of delta-6-desaturase with aging in man, especially on organ-specific decline, and the data available nowadays are clearly insufficient.

Effects on cell membranes

A large amount of data concerning the effects of (ω-3) and (ω-6) PUFA on cell membranes is now available but, in this brief overview, we will only discuss some of the points[15]. The significance of the heterogeneous distribution of (ω-3) FA among various species of warm-blooded animals and among various organs and tissues within different species is still unknown[72,76]. Why, for instance, are (ω-3) FA so abundant in the nervous system, in retinal photoreceptors and in the human and primate testis? Why is the optimal tissue level of arachidonic acid in different tissues obtained at different levels of intake of linoleic acid?

What is the upper limit for (ω-3) supplementation for the brain? In rats, a fish-oil diet given for 8 weeks increased the (ω-3) series and decreased the (ω-6) series in the brain. The effects of this supplementation can be different on the liver: 10% fish oil in the diet increased the (22:6 (ω-3)) FA content of the liver, whereas 14% decreased it. The margin is very narrow[7]. In order to assess experimental results correctly, we must also take into account the fact that the consequences of deficiency or supplementation of (ω-3) or (ω-6) FA appear very slowly in the nervous system (over several months).

Finally, what is the effect of (ω-3) FA supplementation of the mother on the foetus and on the newborn, since it increases (ω-3) long-chain PUFA in human milk?

Production of eicosanoids

Up to now, PUFA requirements have been evaluated in relation to symptoms of PUFA deficiency or to the triene/tetraene blood level ratio. A new concept is emerging, according to which PUFA requirements could be studied in terms of eicosanoid biosynthesis[47]. This new concept might account for the fact that (ω-3) FA seem lees essential than (ω-6) FA, as they give rise less rapidly to eicosanoids.

Dietary intake of (ω-3) and (ω-6) FA changes the PUFA content of the non-essential fatty acid (NEFA) pool and the ratio of highly unsaturated fatty acids (HUFA). Secondarily to the rhythmicity of food intake, the (ω-3) and (ω-6) ratio varies according to a circadian rhythm. In the pool of NEFA in the cells, the ratio of eicosanoid precursors and antagonists varies in a similar way[47]. (ω-6) FA are more quickly oxidized by cyclooxygenase than (ω-3) FA. The rate of synthesis of (ω-6) prostaglandins can be slowed by their precursor dihomogammalinoleic acid, or by the (ω-3) fatty acid eicosapentaenoic acid (timmodonic acid), an alphalinoleic acid derivate, or, as recently demonstrated, by two docosapolyenoic acids: adrenic acid (docosatetraenoic acid) and its derivate (docosapentaenoic acid)[44]. These various inhibiting actions reduce the potential production of arachidonic metabolites so that the latter can be more rapidly inactivated[44,46,48].

Dietary manipulations with various fats produce changes in the following: after 48 h, in the blood spectrum of fatty acids and thromboxane A_2 production; after 7 days, in the urinary excretion of prostanoid metabolites; after 8 weeks, in the phospholipid FA composition and prostaglandin production in tissue and organs[17].

The effects in man of a high (ω-3) FA intake on this subtle regulation, and the physiological significance of the numerous metabolic interactions between eicosanoids, are still unknown. In a normal state, the biosynthesis of eicosanoids in the tissues is suppressed and a burst of biosynthesis occurs only when provoked by a stimulation (when oxygenase activities are stimulated by hydroperoxides)[47].

Some in vivo and in vitro experimental data support the hypothesis that the production of eicosanoids declines with age. The ability to produce prostaglandins decreases with age in rats[13]; prostaglandin I_2 is diminished in aged cultures of human endothelial cells[60] or of smooth muscle rat cells[13]; the ability of the vascular tissue to produce prostacyclin is reduced with age in the pig[41]. Experimental data on the synthesis of thromboxane A_2 in aging are contradictory[3,85]. The loss of the ability of prostaglandin-synthetase to convert free arachidonic acid is probably one of the causes of this decline, since the stimulation of aged cells by ascorbic acid restores this capacity to the level of that of young cells[59,60]. There could also be a shift in the production of prostaglandins with a 50% reduction of prostaglandins (I_2 and E_2), and a doubling of the synthesis of thromboxane A_2 and prostaglandin $PGF_{2\alpha}$[59,60].

Metabolic effects

The (ω-3) and (ω-6) FA have numerous effects on metabolic activities. We have chosen to review those where there are still open questions.

Glucose metabolism

(ω-3) FA seem to increase the sensitivity of tissues to insulin in man[77] and in rat[86]. However, the oral glucose tolerance test is not modified in man by (ω-3) FA, whereas the IV glucose tolerance test shows that the insulin response increases without changing the shape of the blood glucose curve[1], which seems to disagree with the preceding data. In man, the addition of (ω-3) FA (eicosapentaenoic and docosahexaenoic acids) to the test-meal significantly increases the blood and insulin response curve in comparison with that obtained using (ω-6) PUFA or saturated fatty acids; it also increases, although not significantly, the secretion of gastric intestinal polypeptide (GIP)[49].

The effects of (ω-6) PUFA on glucose metabolism are due to the action of arachidonic acid metabolites[23,42,66,67]. It is widely accepted that arachidonic acid metabolites do not change basal insulin levels in man. Prostaglandins of the E_1 group (PGE_1 and E_2) inhibit glucose-induced insulin secretion, but PGE_1 increases the first phase of insulin secretion stimulated by non-glucose secretagogues like arginine[56,57]. Arachidonic acid metabolites are produced in the pancreatic islets. Products that are able to inhibit cyclooxygenase induce insulin secretion and improve glucose homeostasis, while those that inhibit the lipoxygenase reduce insulin secretion resulting from the action of glucose and most other secretagogues[66]. It therefore appears that (ω-3) and (ω-6) PUFA act more like fine modulators of glucose metabolism and insulin secretion than like strong insulin secretagogues.

Lipid metabolism

The literature on the effects of PUFA on lipid metabolism is extensive. For the past 40 years, attention has been focused on (ω-6) FA, and more recently on (ω-3) PUFA. It is accepted that (ω-6) FA, mainly gamma linolenic acid, decrease blood cholesterol levels specifically in LDL, more weakly in HDL and VLDL, and reduce the blood levels of Apo B and Apo A. Several mechanisms have been proposed to account for this hypolipidemic action. These mechanisms can operate simultaneously, but it seems that their relative importance varies with the type of lipid disorder involved. The most important mechanism seems to involve changes in

the fluidity of lipoproteins and cell membranes, although further research is needed to clarify these points.

The hypolipidemic effect of (ω-3) FA has been extensively studied. (ω-3) FA decrease the synthesis of VLDL[18,68]. Eicosapentaenoic and docosahexaenoic acid seem to have the strongest effects. Linolenic acid is less potent. A slight increase in HDL_2 cholesterol is related to docosahexaenoic acid and a high intake of this acid can increase HDL levels. LDL cholesterol is unaffected by these acids and by linolenic acid, and the reduction in blood cholesterol is due to the decrease in VLDL[71]. However, in hypertriglyceridemic patients this effect wanes after one month. As for (ω-6) PUFA, additional mechanisms contribute to the hypolipidemic effect of (ω-3) FA[11,21,24,27,69,87]. We now have a good knowledge of the hypolipidemic effects of (ω-3) and (ω-6) PUFA; however, further human research is needed in order to evaluate the intake of these PUFA that is right for the maintenance of normal blood lipid levels but is free of deleterious side-effects.

Haemorheological effects

The beneficial haemorheological effects of (ω-6) FA have been well demonstrated for several years[24,52,62,64,70]. Although we understand the principle mechanisms of this action, many problems remain unsolved: the modulation of this effect by the quantity of (ω-6) FA intake, the interactions between the various blood cells, the physiological significance of the lipoxygenase pathway and of phospholipid transacylation during activation[43]. More recently, a large number of studies have dealt with the haemorheological effect of (ω-3) FA[11,24,28,35,63,87]. The FA increase the fluidity of blood[20] by changing the deformability of red blood cells, probably because they are incorporated into the cell membrane; the fall in plasma viscosity would be due to changes in lipid and fibrinogen[34]. The synthesis of thromboxane A_2 is reduced because alpha linolenic acid inhibits the synthesis of arachidonic acid from linolenic acid, and the production of eicosanoids from arachidonic acid, by competing for cyclooxygenase. Alpha linolenic acid also competes with arachidonic acid for its incorporation into the 2-position of the phosphoglyceride molecule (where the cyclooxygenase substrates are located)[11]. Despite an impressive amount of research, the precise mechanisms of action of (ω-3) PUFA on blood rheology remain unclear, as does the modulation of their regulation in relation to the (ω-3) FA intake.

Other unresolved and controversial problems remain concerning the physiological effects of (ω-3) and (ω-6) FA: their action on the immune system, on specific organs and tissues such as the heart, the arteries, the kidney, the nervous system and the skin, and also their possible side effects on the biliary tract and their putative promotion of cancer.

The data reported and discussed above show that a large amount of additional research is necessary before we can justify dietary advice to the general population and before we can safely use these fatty acids in therapeutic diets.

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Supraphysiological dosages of vitamins and their implications in man

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Summary. Some recent evidence on the benefits and hazards of elevated dosages of vitamins is summarized. Special emphasis is given on the safety of vitamins A, D, K_1 and B_6. Furthermore, the possibly beneficial effects of vitamins for athletic performance as well as the preventive potential of antioxidative vitamins and of carotenoids against cancer are discussed.
Key words. Vitamins; antioxidative vitamins and cancer; vitamins and athletic performance; carnitine; toxicity of vitamins A, E, K_1, B_6.

Everyone knows that vitamins are essential for health and that a good supply is ensured if we eat the right food. Because people are often uncertain whether they are really getting enough of each vitamin, they tend to supplement their food with high-dosage vitamin pills or capsules. In addition, many vitamins have become known as having additional, beneficial effects. Even 'magic' properties have been claimed for them, if they are taken in large dosages. The question, therefore, arises: How much is enough, and what happens if large dosages are taken up – are they beneficial or toxic? In order to answer those questions, Stähelin, Brubacher and myself organized a symposium on this topic in 1987, and this paper is based on the symposium book issued in 1989. It will, however, not be possible to give a complete overview. After a short general introduction, I will mainly discuss the oral uptake of supraphysiological dosages by healthy adults, and will not include clinical cases where the supplementation with vitamins is of importance. It will also not be possible to cover all the vitamins.

Recommended dietary allowances

Before discussing supraphysiological dosages, it is necessary to give some thought to the difficulty of determining the so-called recommended dietary allowance (RDA). Each vitamin has many functions in our body. When the daily intake is reduced, the most sensitive function of each vitamin will be affected first, and if the insufficient uptake is continued, further functions will be impaired. Finally, the first signs of the specific vitamin deficiency disease will appear[4]. In order to make a recommendation for the daily intake we have to know the minimal requirement for maximal protection for each vitamin-dependent function. This *functional requirement* shows a large individual variation, and a *safety margin* has to be defined when a country sets its RDA values. It has, furthermore, to be taken into consideration that all vitamins can be stored in our bodies, but the storage capacity for each vitamin is different. We therefore also have to take into account a *storage requirement*, i.e. a minimal requirement to allow for adequate storage pools of each vitamin.