

## The metabolism and availability of essential fatty acids in animal and human tissues

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**Summary** — Essential fatty acids (EFA), which are not synthesized in animal and human tissues, belong to the n-6 and n-3 families of polyunsaturated fatty acids (PUFA), derived from linoleic acid (LA, 18:2n-6) and  $\alpha$ -linolenic acid (LNA, 18:3n-3). Optimal requirements are 3–6% of ingested energy for LA and 0.5–1% for LNA in adults. Requirements in LNA are higher in development. Dietary sources of LA and LNA are principally plants, while arachidonic acid (AA, 20:4n-6) is found in products from terrestrial animals, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in products from marine animals. EFA are principally present in dietary triacylglycerols, which should be hydrolyzed by lipases in gastric and intestinal lumen. DHA seems to be released more slowly than the others. Its intestinal absorption is delayed but not decreased. Long-chain PUFAs are incorporated in noticeable amounts in chylomicron phospholipids. However, their uptake by tissues is no more rapid than uptake of shorter chain PUFA. In tissues, LA and LNA, which constitute the major part of dietary EFA, should be converted into fatty acids of longer and more unsaturated chain by alternate desaturation ( $\Delta 6$ ,  $\Delta 5$ ,  $\Delta 4$ )—elongation reactions. Animal tissues are more active in this biosynthesis than human tissues. Liver is one of the most active organs and its role is critical in providing less active tissues, particularly the brain, with long-chain PUFA secreted in VLDL (very low density lipoprotein). In liver, many nutritional, hormonal and physiological factors act on the PUFA biosynthesis. Dietary fatty acids exert a great influence and are often inhibitory. Dietary LNA inhibits  $\Delta 6$  desaturation of LA. The desaturation products AA, EPA, and DHA inhibit  $\Delta 6$  desaturation of LA and  $\Delta 5$  desaturation of DGLA (dihomo- $\gamma$ -linolenic acid). With regard to hormones, insulin and thyroxin are necessary to  $\Delta 6$  and  $\Delta 5$  desaturation activities, whereas other hormones (glucagon, epinephrine, ACTH, glucocorticoids) inhibit desaturation. Concerning the physiological factors, the age of individuals is critical. In the fetus, the liver and the brain are capable of converting LA and LNA into longer-chain EFA, but these are also delivered by the mother, after synthesis in the maternal liver and placenta. Just after birth, in animals, the  $\Delta 6$  desaturation activity increases in the liver and decreases in the brain. In aging, the capacity of the whole liver to desaturate LA and DGLA is equal at 1.5 and 25 months of age in rats fed a balanced diet throughout their life and the AA and DHA content of tissue phospholipids is unchanged in aging. Fatty acid oxidation in the liver is also likely to decrease the availability of EFA in tissues, in

particular oxidation of long-chain PUFAs which is high in peroxisomes. However, in healthy individuals, despite the numerous factors likely to influence the availability of EFA, in most cases a regular and balanced diet meets the tissue requirements in n-6 and n-3 EFA

**essential fatty acids / dietary sources / desaturation / oxidation / nutritional factors / hormonal factors / physiological factors**

**Résumé — Métabolisme et disponibilité des acides gras indispensables dans les tissus animaux et humains.** Les acides gras indispensables (EFA), qui ne sont pas synthétisés dans les tissus animaux et humains, appartiennent aux 2 familles d'acides gras polyinsaturés (PUFA) n-6 et n-3, dérivant de l'acide linoléique (LA, 18:2n-6) et de l'acide  $\alpha$ -linoléique (LNA, 18:3n-3). Les besoins optima sont de 3–6% de l'énergie ingérée pour LA et de 0,5–1% pour LNA chez les adultes. Les besoins en LNA sont supérieurs au cours du développement. Les sources alimentaires de LA et de LNA sont principalement végétales, tandis que l'acide arachidonique (AA, 20:4n-6) est présent dans les produits des animaux terrestres et les acides eicosapentaénoïque (EPA) et docosahexaénoïque (DHA) sont présents dans les produits des animaux marins. Ces acides gras sont présents principalement dans les triacylglycérols, qui doivent être hydrolysés par des lipases dans la lumière gastrique et intestinale. Le DHA paraît libéré plus lentement que les autres. Son absorption intestinale est retardée mais non diminuée. Les PUFA à longue chaîne sont incorporés dans les phospholipides des chylomicrons en proportion notable. Toutefois, ils ne sont pas captés par les tissus plus rapidement que les PUFA plus courts. Dans les tissus, LA et LNA, qui constituent la majeure partie des EFA fournis par l'alimentation, doivent être convertis en acides gras à plus longue chaîne et plus insaturés par une série alternée de désaturations ( $\Delta 6$ ,  $\Delta 5$  et  $\Delta 4$  désaturations) et d'élongations. Dans cette biosynthèse, les tissus animaux sont plus actifs que les tissus humains. Le foie est un des organes les plus actifs et son rôle est primordial pour l'approvisionnement des autres organes moins actifs, comme le cerveau, en PUFA à longue chaîne sécrétés avec les VLDL (very low density lipoprotein). Dans le foie, de nombreux facteurs nutritionnels, hormonaux et physiologiques interviennent sur la biosynthèse des PUFA. Les acides gras du régime ont une grande influence et sont souvent inhibiteurs. Ainsi LNA alimentaire inhibe la  $\Delta 6$  désaturation de LA. De même, AA, EPA et DHA, produits des désaturations, inhibent la  $\Delta 6$  désaturation de LA et la  $\Delta 5$  désaturation de DGLA (dihomo- $\gamma$ -linoléique). Parmi les hormones, l'insuline et la thyroxine sont nécessaires à l'activité des  $\Delta 6$  et  $\Delta 5$  désaturation tandis que d'autres hormones (glucagon, adrénaline, ACTH, glucocorticoïdes) les inhibent. Concernant les facteurs physiologiques, l'âge est un facteur important. Chez le fœtus, le foie et le cerveau sont capables de convertir LA et LNA en EFA plus longs mais ceux-ci sont aussi apportés par la mère, synthétisés dans le foie et le placenta maternels. Juste après la naissance, chez l'animal, l'activité de  $\Delta 6$  désaturation augmente dans le foie et diminue dans le cerveau. Au cours du vieillissement, les capacités du foie entier à désaturer LA et DGLA sont les mêmes à 1,5 et 24 mois chez le rat recevant un régime équilibré toute sa vie, et les contenus en AA et DHA des phospholipides tissulaires ne sont pas modifiés avec l'âge. L'oxydation des acides gras dans le foie peut aussi diminuer la disponibilité en EFA dans les tissus en particulier l'oxydation des PUFA à longue chaîne qui est élevée dans les peroxisomes. Toutefois, chez les individus sains, en dépit des nombreux facteurs susceptibles d'influencer la biodisponibilité en EFA, un régime régulier et équilibré permet, dans la plupart des cas, de satisfaire les besoins des tissus en EFA n-6 et n-3.

**acides gras indispensables / sources alimentaires / désaturations / oxydation / facteurs nutritionnels, hormonaux et physiologiques**

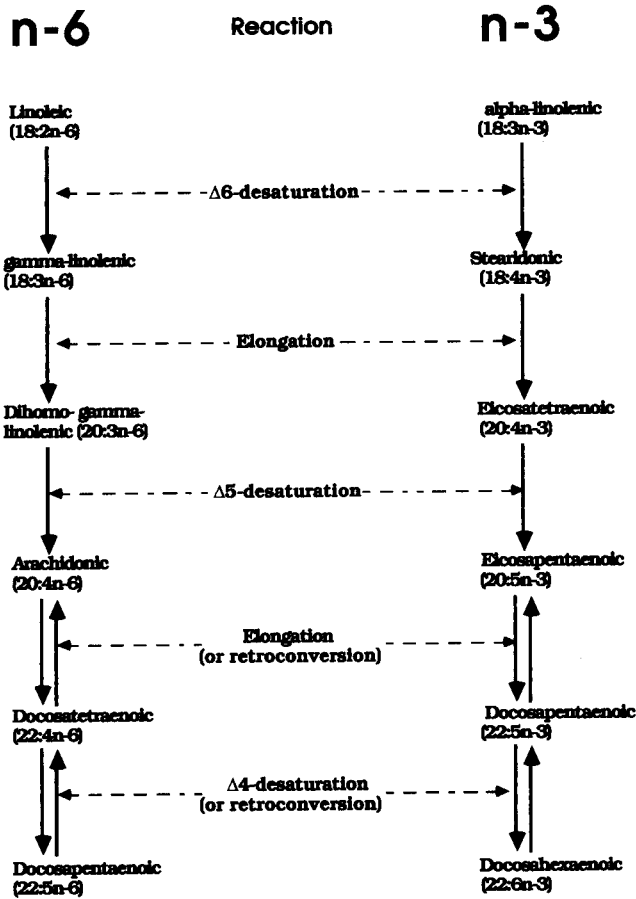
**INTRODUCTION**

The properties of lipids are those of their component fatty acids. These can be divided, according to their chemical structure, into 2 broad groups: saturated and

unsaturated fatty acids. The latter can then be further sub-divided, according to their biological properties, into 2 different narrower groups: mono- and polyunsaturated fatty acids (PUFA). The latter group is composed of 4 families of fatty acids. The first 2

are the palmitoleic (n-7) and oleic (n-9) families which are not essential since they are synthesized *de novo* in most cells. On the contrary, the next 2 families, namely the linoleic (n-6) and linolenic (n-3) families, cannot be synthesized *de novo* by animal cells and hence must be provided in the diet. All the members of these 2 independent families derive from their respective precursors, linolenic acid (LA, 18:2n-6) and  $\alpha$ -linolenic acid (LNA, 18:3n-3) by alternate desaturation–elongation reactions (fig 1) (Sprecher, 1981).

In the absence of these dietary unsaturated n-6 and n-3 fatty acids, growth, learning, visual activity are impaired in rats and severe structural and metabolic disorders take place (Burr and Burr, 1929; Holman, 1970; Neuringer *et al*, 1988; Vergroesen and Crawford, 1989). These perturbations underline the important functions of essential fatty acids (EFA). Indeed EFA are important in membrane structure as major components of phospholipids for the integrity and fluidity of intracellular and plasma mem-



**Fig 1.** Outline of the more common pathways by which unsaturated fatty acids of the n-6 (linoleic) and n-3 (linolenic) families are converted to long-chain polyunsaturated fatty acids in animal tissues.

branes (Kinsella, 1991). In this regard, they modulate activity of membrane-bound receptors, enzymes, molecule carriers and ionic channels. In addition, several of them, namely dihomo- $\gamma$  linolenic (DGLA, 20:3n-6), arachidonic (AA, 20:4n-6) and eicosapentaenoic (EPA, 20:5n-3) acids can be easily converted into a wide range of eicosanoids including prostanoids (prostaglandins), thromboxanes, prostacyclines and leukotrienes. These have potent biological effects regulating such important physiological functions as reproduction, heart and vascular physiology and immune functions (Curtis-Prior, 1988). Finally, n-6 fatty acids are often associated with cholesterol in plasma (18:2 and/or 20:4n-6) and in adrenals (adrenic acid, 22:4n-6) thereby participating in the normal intravascular and cellular metabolism of this key molecule.

To help their important structural and biological functions, EFA must be provided to tissues in appropriate and equilibrated amounts. Many factors are likely to affect their availability. Their dietary intake is one of the most critical.

## REQUIREMENTS IN ESSENTIAL FATTY ACIDS

Recommended dietary intakes of essential fatty acids are not easy to be proposed, since optimal requirements are not fully known. However, some information is available (Lemarchal *et al*, 1992).

In the n-6 family, LA is the major essential fatty acid and no more than 1–2% of calories is required to relieve symptoms of essential fatty acid deficiency in rats (Kinsella, 1991). But to ensure at best the biological functions of essential fatty acids, intake of 3–6% is generally recommended. Higher amounts have sometimes been proposed in adults, since LA is beneficial in reducing LDL-cholesterol, atherosclerosis

and coronary heart disease. But recent results are indicative of a positive relationship between LA intake and coronary heart disease (Hodgson *et al*, 1993). It seems prudent, therefore, not to promote increased consumption of this essential fatty acid. An intake of 7–10 g LA per day seems reasonable. On the other hand, intake of AA probably decreases the need for LA, but the optimum ratio between these 2 essential n-6 fatty acids is not known.

LNA, the precursor of EPA and docosahexaenoic acids (DHA) is also required, especially in the diet of pregnant women and nursing mothers as a source of DHA for neural and visual tissues during development of the embryo and neonate (Kinsella, 1991). From results of different authors (Holman *et al*, 1982; Bjerve *et al*, 1987; Bourre *et al*, 1989; Guesnet *et al*, 1993), the optimal requirement for LNA could be 0.5–1% of calories, while requirements for EPA and DHA would be 0.4%. According to Bjerve *et al* (1989), the optimal intake of LNA would be 860 to 990 mg per day, and 350–400 mg for EPA and DHA. The optimal balance between EPA and DHA was not proposed by these authors.

Since DHA is a major fatty acid component of the grey matter of the cerebral cortex and of the photoreceptor membranes of the rod outer segment of the retina and since deprivation of LNA has been associated with impaired learning and visual activity (Neuringer *et al*, 1988), the need for n-3 essential fatty acids has been estimated to be higher in the early stages of life, to ensure optimum development and function of neural and visual tissues, *ie* 0.7–1.2% of calories according to Lemarchal *et al* (1992).

A general agreement is that intake of n-6 and n-3 essential fatty acids ranging from 4:1 to 6:1 is prudent (Neuringer *et al*, 1988). Most human milks analyzed contain around 1–2% n-3 fatty acids and the n-6/n-3 fatty acid ratio is within the range suggested. It may reflect the need of the neonate for opti-

mal growth, depending on tissue AA content (Carlson *et al*, 1992) and optimal neural and visual development, depending on tissue DHA content (Uauy *et al*, 1992).

## DIETARY SOURCES OF ESSENTIAL FATTY ACIDS

As already mentioned, since they cannot be synthesized *de novo*, n-6 and n-3 polyunsaturated fatty acids must be provided in the diet. Plants are the principal sources of these fatty acids. Unlike animals, plants possess the capacity to insert double bonds between the existing n-9 double bond and the methyl end of oleic acid (Demandre *et al*, 1986). Edible vegetable oils, such as sunflower, corn or peanut oil, constitute a major source of LA (Kinsella, 1991). Table I shows that 65% of the sunflower oil fatty acids are LA. The amount is still higher in safflower oil (75–80%). In addition, several oils provide LNA, soybean oil (8%), low-erucic acid rapeseed oil (9%) and walnut oil (13%). The

chloroplast membranes of green leafy plants are very rich in LNA but the total amount of the acid provided by these foods is modest since they do not contain large amounts of lipids (Kinsella, 1991). Animal feeds generally contain low amounts of LA. Human milk supplies the neonate with a relatively high quantity of LA (10–13%) (Martin *et al*, 1993). On the contrary, bovine milk and the dairy products are poor sources of essential fatty acids (Maniongui *et al*, 1991). Longer-chain essential n-6 and n-3 fatty acids are generally provided in food from animal sources, *ie* liver, kidneys, eggs, whereas marine foods are good sources of EPA and DHA (n-3). Fresh water fish contains both n-6 and n-3 very long-chain fatty acids (Kinkela and Bézard, 1993). Human milk also provides the neonate with very long-chain essential n-6 and n-3 fatty acids (Martin *et al*, 1993). Several vegetable oils contain essential fatty acids located beyond the rate-limiting  $\Delta$ 6-desaturation step. Evening primrose, borage and blackcurrant oils contain significant amount of  $\gamma$ -linolenic acid (GLA, 18:3n-6).

**Table I.** Examples of n-6 and n-3 essential fatty-acid concentration (mol %) in lipids of some animal products and edible oils.

	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Human milk <sup>a</sup>	12.7	0.6	1.0 <sup>b</sup>		0.4 <sup>b</sup>
Bovine milk <sup>c</sup>	1.9	0.8			
Beef fat <sup>d</sup>	5	1			
Pork fat <sup>e</sup>	6	2			
Pork liver (TAG) <sup>f</sup> <sup>e</sup>	17	1	4	1	1
Egg yolk (TAG) <sup>f</sup> <sup>g</sup>	10	0.6			
Egg yolk (PL) <sup>f</sup> <sup>h</sup>	16	0.1	3.8		1.9
Sunflower oil <sup>i</sup>	65	0.2			
Peanut oil <sup>i</sup>	33	0.1			
Rapeseed oil <sup>i</sup>	22	9			
Walnut oil <sup>i</sup>	59	13			
Sea fish <sup>i</sup>	2	1	1	12	39
Fresh-water fish <sup>i</sup>	8	2	9	8	13

<sup>a</sup> Martin *et al*, 1993; <sup>b</sup>  $\Sigma$ n-6 and  $\Sigma$ n-3 very-long-chain fatty acids; <sup>c</sup> Maniongui *et al*, 1991; <sup>d</sup> Brockerhoff *et al*, 1966; <sup>e</sup> Christie and Moore, 1970a; <sup>f</sup> TAG = triacylglycerols, PL = phospholipids; <sup>g</sup> Christie and Moore, 1970b; <sup>h</sup> Kuksis and Marai, 1967; <sup>i</sup> Commercial samples, personal analyses, unpublished data; <sup>j</sup> Kinkela and Bézard, 1993.

Blackcurrant oil also contains stearidonic acid (18:4n-3) (Lawson and Hughes, 1988a).

From the above data, it is evident that a varied diet in sufficient amounts can easily provide n-6 and n-3 essential fatty acids in suitable amounts. However, the dietary fatty acids must first cross the intestinal epithelium before they can reach the different tissues. Digestion and absorption of dietary lipids constitute further factors that can influence availability of EFA.

### DIGESTION AND INTESTINAL ABSORPTION OF ESSENTIAL FATTY ACIDS

A recent report (Carlier *et al*, 1991) dealt with the question of digestion and absorption of EFA. A previous review (Nelson and Ackman, 1988) had been principally concerned with n-3 PUFA.

Dietary fatty acids are mainly present in triacylglycerols (> 95%). The others are provided by phospholipids and cholesteryl esters, the dietary intake of which is important since they contain high proportion of very long-chain EFAs.

The processes of lipid digestion and absorption have been reviewed several times in the last few years (Bézard and Bugaut, 1986; Bernard and Carlier, 1989; Thomson *et al*, 1989; Carlier *et al*, 1991).

With regard to PUFA, 2 peculiarities have to be considered, *ie* their structure which determines their chemical and biological properties and their position in dietary triacylglycerol and phospholipid molecules.

Concerning the structure of n-3 polyunsaturated fatty acids, several authors (Brockhoff and Yurkowski, 1966; Bottino *et al*, 1967) have observed that certain long-chain PUFA of marine oils were resistant *in vitro* to pancreatic lipase hydrolysis. This was attributed to the presence of a double bond between carbon 2 and carbon 5 of the acyl chain (when counted from the carboxylic

end). This is the case with DHA, in which the first double bond is located at the 4th carbon atom. The resistance to lipolysis seemed independent of the position of fatty acid in the glycerol moiety of triacylglycerol. This property could impair absorption of DHA from marine oils when transposed *in vivo*. However, this point is very controversial and many studies *in vivo* gave conflicting results (Carlier *et al*, 1991). It seems that absorption could be delayed but not decreased, so that availability would not be affected. Nevertheless further investigations are required to answer this question.

Another problem is the digestion of EPA and DHA when supplemented as ethyl esters (instead of natural glyceryl esters). Their absorption is impaired (Lawson and Hughes, 1988b). However, hydrolysis takes place, probably less rapidly but completely. The absorption could be impaired because of lack of the 2 monoacyl-sn-glycerols necessary for triacylglycerol resynthesis in enterocytes. Indeed absorption was considerably increased when ethyl esters were accompanied by dietary triacylglycerols (Lawson and Hughes, 1988c; Yang *et al*, 1989).

The position of fatty acids in the triacylglycerol molecules is another factor affecting their hydrolysis and absorption. Triacylglycerols are partially hydrolysed in the stomach in man in the presence of a gastric lipase. It preferentially hydrolyzes fatty acids in the external sn-1 and sn-3 positions, with the sn-3 more rapidly cleaved than the sn-1 position (Ransac *et al*, 1990, Rogalska *et al*, 1990). Lipolysis continues in the small intestine catalyzed by pancreatic lipase which splits off fatty acids in sn-1 and sn-3 positions at equal rate. The 2 monoacyl-sn-glycerols formed are rapidly absorbed, while the liberated fatty acids are likely to form insoluble calcium soaps and to be absorbed to a lower extent. This underlines the importance of the EFA position in dietary triacylglycerols.

The data in table II confirm the well-known preferential esterification of LA in

sn-2 position in vegetable oils (Litchfield, 1972) and even in pork liver triacylglycerols, whereas it is not true in depot fats (Christie and Moore, 1970b). In humans, the coefficient of digestibility of the most common vegetable oils is 97–98%. Only hydrogenated oils, comprising high amounts of saturated and isomeric fatty acids, have lower coefficients (Deuel, 1955). In these conditions, LA can be considered as totally absorbed. Contrary to LA, LNA is preferentially esterified in external positions in soybean oil triacylglycerols (table II). However, its absorption is probably total since the coefficient of digestibility of soybean oil was found to be 97% in humans, and 98% in rats (Deuel, 1955). EPA and DHA are much more often found in sn-2 position in herring oil (table II) as in other fish oils (Brockhoff *et al.*, 1968). The only problem is the possible resistance of DHA to lipoly-

sis, not its location in fish oil triacylglycerols.

How and to what extent are fish-oil fatty acids absorbed in the small intestine? Conflicting results were obtained as for the amount absorbed (Carlier *et al.*, 1991). According to results obtained on rats fed 0.5 ml corn oil, menhaden oil or cod liver oil, no difference was observed between oils concerning the amount absorbed (Carlier *et al.*, 1991). The only difference was that cod liver oil absorption was delayed, since peak absorption occurred at 5 h instead of 2–3 h for the other 2 oils. The difference could be accounted for by the higher content in DHA of cod liver oil, when compared to menhaden oil, while the reverse was true for EPA. Other differences in fatty acid composition also exist between the 2 fish oils, which could also explain the different absorption profiles.

**Table II.** Distribution of fatty acids between the 3 positions of the glycerol moiety of triacylglycerols from several foods.

	Position <sup>a</sup>	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Peanut oil <sup>b</sup>	1, 2, 3	32				
	1	32				
	2	56				
	3	12				
Soybean oil <sup>c</sup>	1, 2, 3	54	8			
	1	29	38			
	2	43	29			
	3	28	33			
Pork liver <sup>d</sup>	1, 2, 3	17		4		
	1	14		13		
	2	48		31		
	3	38		55		
Herring oil <sup>e</sup>	1, 2, 3	2			8	5
	1	43			12	7
	2	43			72	86
	3	14			16	7

<sup>a</sup> 1, 2, 3: percentage of fatty acid in total triacylglycerols; 1-2-3: percentage of fatty acid in the 3 positions (total = 100);

<sup>b</sup> Sempore and Bézard, 1991; <sup>c</sup> Yurkowski and Brockhoff, 1966; <sup>d</sup> Christie and Moore, 1970a; <sup>e</sup> Brockhoff *et al.*, 1968.

An interesting observation by several authors (Chen *et al*, 1985; Carlier *et al*, 1991) is the higher recovery of PUFA in phospholipids in comparison with oleic acid. In rats, 18% of 20:4n-6 was incorporated into lymph phospholipids, instead of 3% of oleic acid in the 3rd half-hour following absorption of the free fatty acids in rats (Carlier *et al*, 1991). Several explanations can be provided. Because of selectivity of acyl-transferases, generally AA, EPA and DHA are rapidly and selectively acylated into phospholipids (Kinsella, 1991). Secondly, the L-FABP (liver-fatty acid binding protein) present in enterocytes, contrary to the I-FABP (intestinal-FABP), might exhibit higher affinity for PUFA than for others (Lowe *et al*, 1987) and might thus channel these acids to phospholipid synthesis.

Dietary phospholipids are hydrolyzed in the presence of pancreatic phospholipase A2 which splits off the fatty acids esterified in the sn-2 position. The cholesterol ester hydrolase hydrolyzes cholesteryl esters. The hydrolysis products together with those derived from triacylglycerols are utilized in the enterocytes to form chylomicrons, which are secreted in the lymph.

Besides their function of transferring fatty acids from the intestinal lumen to the lymph, enterocytes can also metabolize EFA. Bernard *et al* (1991) have shown that, when rats were fed diets containing (1-<sup>14</sup>C) LA, 4–7% of the radioactivity recovered in lymph lipids was present in AA. Caselli *et al* (1993) have further demonstrated that the intestine was itself able to synthesize and secrete AA from dietary LA.

### UPTAKE OF ESSENTIAL FATTY ACIDS BY TISSUES

The absorbed EFA are taken up by tissues. Although PUFA are recovered in phospholipids in relatively high proportion, it does

not seem that they are taken up at higher or lower rates than others, as observed by Chen *et al* (1987). The authors injected recipient rats with lymph chylomicrons containing triglycerides enriched with labelled oleic acid or eicosapentaenoic acid (EPA). During the early stages of uptake, they observed no difference between the disappearance rates of both types of chylomicrons. However, after longer time periods, clearance of EPA-enriched chylomicrons was slightly lower.

Results dealing with the uptake by the different tissues or organs of essential PUFA esterified in triacylglycerols, phospholipids or cholesteryl esters are not abundant. On the contrary, because of easier interpretation, experiments with non-esterified fatty acids are more numerous. For example, Anderson and Connor (1988) have studied the uptake of free labelled fatty acids bound to albumin by the developing brain of 15-day-old rats, in comparison with liver. They observed a steady and significant increase in uptake of labelled fatty acid in the brain as the degree of unsaturation increased. DHA (22:6n-3) showed more than twice as much uptake as palmitic acid (16:0) and considerably more than LA (18:2n-6). The liver showed an even stronger preference for 22:6n-3 than the brain, with the liver accumulating 4 times more radioactivity from this fatty acid than for the other 4 fatty acids tested, which were very similar in the uptakes. Expressed per gram of wet tissue uptake of 22:6n-3 by the liver was 65-fold higher than by the brain.

### AVAILABILITY OF ESSENTIAL FATTY ACIDS IN TISSUES

The essential fatty acids used by animal cells to incorporate into their membrane phospholipids are most often long-chain PUFA, for example, AA (20:4n-6) and DHA (22:6n-3). The C20 polyunsaturated fatty



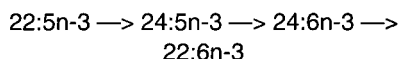
acids (20:3n-6, 20:4n-6 and 20:5n-3) are used to synthesize eicosanoids. In cells or in plasma, cholesterol is frequently associated with n-6 PUFA (20:4n-6, 22:4n-6) to form cholesteryl esters. However, diet provides essential fatty acids principally as LA and LNA. In tissue, these precursors should be converted into longer and more unsaturated fatty acids.

The conversion of the 2 precursors requires an alternating sequence of chain desaturation ( $\Delta 6$ ,  $\Delta 5$  and  $\Delta 4$  desaturations) and elongation reactions (fig 1) (Sprecher, 1981). These reactions, which occur principally on endoplasmic reticulum membranes (Brenner, 1989), require activated acyl-CoA esters. The activation is rapid and not rate limiting (Naughton, 1981). Desaturation introduces a double bond proximal to the carboxyl group, so that the molecules successively formed retain the position of the first double-bond in relation to the methyl end of the carbon chain, hence the 'n' (or  $\omega$ ) designation. The first desaturation step in the biosynthesis of longer chain polyenic fatty acids is  $\Delta 6$ -desaturation, effected by the enzyme  $\Delta 6$ -desaturase. The same enzyme converts LA to GLA and at a higher rate LNA to stearidonic acid. It is the rate-limiting reaction in the synthesis of AA and EPA (Marcel *et al*, 1968). Since both LA and LNA are substrates of the same enzyme, competition occurs between the essential 2 fatty acids (Sprecher, 1989). Too high an amount of LNA in the diet can decrease conversion of LA into higher polyunsaturated n-6 fatty acids. Conversely too high an intake of LA is likely to impair synthesis of EPA and DHA from LNA. An appropriate dietary n-6/n-3 fatty acid ratio is therefore critical.

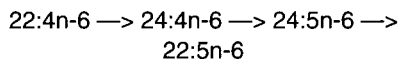
A second enzyme, the  $\Delta 5$ -desaturase, converts dihomo- $\gamma$ -linolenic acid (DGLA) to arachidonic acid (AA). Both fatty acids, substrate and product, are precursors of eicosanoids of families 1 and 2. Activity of the  $\Delta 5$ -desaturase activity, regulates the

availability of each precursor.  $\Delta 5$ -desaturase also converts 20:4n-3 to 20:5n-3 (EPA), the precursor of the 3rd family of eicosanoids. This shows the importance of the  $\Delta 5$ -desaturation step. The  $\Delta 5$ -desaturase, under certain circumstances, may be a rate-limiting enzyme (Bernert and Sprecher, 1975).

The  $\Delta 4$ -desaturation step necessary to form 22:6n-3 may not require a specific  $\Delta 4$ -desaturase enzyme. Voss *et al* (1991) have shown that conversion of 22:5n-3 to 22:6n-3 involved not one but 3 reactions, namely elongation followed by  $\Delta 6$ -desaturation in microsomes, and a peroxisomal shortening according to the scheme:



This revised pathway for 22:6n-3 biosynthesis has also recently been evidenced by Geiger *et al* (1993) for the n-6 family, according to the scheme:



Cook *et al* (1991) have described an alternative pathway of 18:3n-3 conversion into 20:5n-3 observed in rat glioma cells. Though controversial, the synthesis would involve an elongation step followed by 2 desaturation steps,  $\Delta 5$ - and  $\Delta 8$ -desaturation.  $\Delta 6$ -desaturase would not be implicated in this pathway.

Hagve and Christophersen (1986) have shown in isolated liver cells that adrenic acid (22:4n-6) and docosahexaenoic acid (22:6n-3) can be shortened by peroxisomal  $\beta$ -oxidation and that this reaction can be used to retroconvert the products to arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3), respectively. By this means, a relatively high amount of 22:6n-3 in phospholipids can generate 20:5n-3 in low amounts, for biosynthesis of the third series of prostanoids.

Biosynthesis of longer chain polyenic fatty acids also proceeds through elongation steps resulting in addition of a 2-carbon unit to the acyl chain proximal to the carboxyl group (Sprecher, 1981). Apparently elongation reactions are not rate-limiting (Bernert and Sprecher, 1975). Elongation and desaturation of 20:4n-6 and 20:5n-3 principally take place in endoplasmic reticulum. However, some transformations were observed to occur in peroxisomes (Mimouni *et al*, 1991).

The capacity of tissues or organs to synthesize n-6 and n-3 long-chain PUFA from LA and LNA, respectively, depends on the presence of active desaturase and elongase enzymes. The relative activity of these enzymes determine the relative amounts of products formed. Brenner (1971) showed that desaturation activity is not evenly distributed in all the organs. He studied the conversion of LA to GLA by  $\Delta 6$ -desaturation in microsomes from different organs. If the activity in liver was taken as 100, the activity found in different organs were: adrenals (142), testicles (45), heart (8), kidneys (12), and brain (14), while no activity was detectable in lung and epididymal fat. In adult mice, Bourre *et al* (1990) showed that  $\Delta 6$  desaturation activity in brain was *ca* 6% that found in liver. Thus, important organs such as brain heart and kidney, greatly depend on the liver for the synthesis and supply of long-chain PUFA.

However, the liver is not the only organ to provide tissues with longer chain fatty acids synthesized from LA. As mentioned above, the small intestine can synthesize and secrete AA from LA (Caselli *et al*, 1993). Placenta may also transform LA to AA. This question will be discussed later. In the appreciation of desaturation activity, the age of animals should also be taken into account, since the activity was observed to vary considerably with aging and differently from an organ to another (Cook, 1991), as will be seen later.

However, despite some adaptations, in particular in the developing brain, organs generally greatly depend on the liver for providing their long-chain PUFA.

For this reason, factors influencing the synthesis of these long-chain fatty acids in the liver were extensively studied. These factors influence the desaturases themselves, which are the rate-limiting steps of the desaturase systems and not the other 2 associated proteins, NADH cytochrome  $b_5$  reductase and cytochrome  $b_5$  (Brenner, 1981).

For simplicity, the factors influencing desaturase activity may be classified into nutritional factors, hormonal factors and physiological factors such as age. But it is evident that these factors are not independent and, in some cases, the effects of nutritional factors are likely to be explained by modification of the hormonal status. The major effects are reported in tables III and IV. Further detailed information is available in previous reviews (Brenner, 1989; Lemarchal, 1989; Kinsella, 1991).

### **Nutritional factors**

Among the nutritional factors, dietary fatty acids are probably the most important (table III). Generally, fat-free diets and those deficient in EFA result in a sharp increase in  $\Delta 6$ -desaturase activity in rat liver. Marcel *et al* (1968) observed that the  $\Delta 6$ -desaturase activity of liver microsomes from rats fed a fat-free diet (deficient in EFA) was 2-fold greater compared with the activity in microsomes from chow-fed rats. Castuma *et al* (1972) and Brenner *et al* (1981) observed the same effect but to a lesser extent. The effects of EFA deficiency on  $\Delta 5$ -desaturation activity are more controversial. De Gomez Dumm *et al* (1983) observed that the activity was decreased. However, contrary to this, several authors observed that

**Table III.** Nutritional factors affecting  $\Delta 6$ - and  $\Delta 5$ -desaturation in rat liver microsomes

	$\Delta 6$	$\Delta 5$
<i>Dietary fatty acids</i>		
Essential fatty acid (EFA) deficiency	+	+ -
$\alpha$ -Linolenic acid (18:3n-3)	-	+
Eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)	-	-
Linoleic acid (18:2n-6)	+	+
$\gamma$ -Linolenic acid (18:3n-6)	+ -	0
Arachidonic acid (20:4n-6)	-	-
Oleic acid (18:1n-9)	-	-
Trans-fatty acids	- 0	+ - 0
Cyclopropenoic fatty acids	-	-
<i>Other factors</i>		
Cholesterol		
<i>In vitro</i>	+	+
<i>In vivo</i>	-	-
Protein		
Low intake	-	-
High intake	+	+
Glucose, fructose, glycerol	-	-
Ethanol	-	-
Zinc deficiency	+ 0	-
High NaCl intake	+ -	+ -
Fasting	-	+ -
Fasting + glucose refeeding	+	-

+ : stimulation; - : inhibition; 0 : no effect; references in the text.

the  $\Delta 5$ -desaturation was increased (Christiansen *et al*, 1969; Do and Sprecher, 1975). Blond and Bézard (1991) showed that in liver microsomes from rats fed a fat-free diet, the conversion of DGLA into AA by  $\Delta 5$ -desaturation was 3-fold higher after 15 min of incubation, compared with the activity of liver microsomes from rats fed a commercial diet. The increase in  $\Delta 6$ - and  $\Delta 5$ -desaturation activity may reflect a response to ensure maximum synthesis of PUFA from 18:1n-9 (n-9 family) to maintain the fluidity of membranes. In this view, Brenner *et al* (1981) demonstrated that changes in fatty-acid composition of the microsomal membranes induced by a diet deficient in EFA did not alter the phase transition tem-

perature of the lipids as determined by electron spin resonance.

The presence of certain fatty acids in the diet is also likely to modify either the activity of the  $\Delta 6$ - or the  $\Delta 5$ -desaturase or the desaturation rate by competition. Sometimes both occur. The best example is the presence of LNA, substrate of the  $\Delta 6$ -desaturase and competitive inhibitor of LA desaturation. As early as 1964, Holman reported that LNA was by far the most potent inhibitor of  $\Delta 6$ -desaturase in the diet or in the incubation medium. When an increasing amount of LNA was added to rat liver homogenates, Blond and Lemarchal (1984) showed that  $\Delta 6$ -desaturation of LA was highly decreased

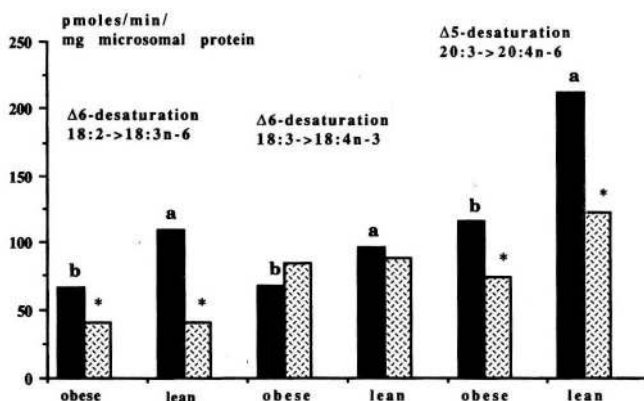
while  $\Delta 5$ -desaturation of DGLA was also decreased but to a lesser extent. This was confirmed by observations *in vivo* by Bourre *et al* (1989). Garg *et al* (1988a) also observed *in vivo* that, when present in the diet, LNA contained in high amounts in linseed oil, also evoked inhibition (by 30%) of  $\Delta 6$ -desaturation when compared to a control diet (beef tallow). On the contrary, the  $\Delta 5$ -desaturation rate was increased (x 1.5) (Garg *et al*, 1988a). However, in similar experiments, Choi *et al* (1989) and Christiansen *et al* (1991) showed that the  $\Delta 6$ -desaturase activity was increased (+50%). The  $\Delta 5$ -desaturase activity was also increased (Christiansen *et al*, 1991) and to higher extent (x 3.5) than was observed by Garg *et al* (1988b). These discrepancies probably originate from differences in the control diet. Garg *et al* (1988a,b) used beef tallow, rich in saturated fatty acids, as a control fat, but at the same amount as the experimental diet (20% wt), whereas Christiansen *et al* (1991) used a commercial diet containing 5% of lipids, compared to 20% of linseed oil in experimental diet. The amount of dietary lipids is likely to influence the desaturation rate as can the type of control fat. Indeed Kurata and Privett (1980) showed that the level of fat affected the composition of liver fatty acids. The AA content increased from 11 to 21% in rats consuming 5 and 20% coconut oil respectively for 33 weeks, apparently indicating that  $\Delta 6$ - and  $\Delta 5$ -desaturases were stimulated by the high fat diet, although differences in AA utilisation may be also involved.

Results obtained with the long-chain n-3 fatty acids, EPA + DHA are less controversial. These acids found mainly in fish oils markedly depress  $\Delta 6$ -desaturase enzyme (Choi *et al*, 1989; Christiansen *et al*, 1991). They also depress  $\Delta 5$ -desaturase activity (Garg *et al*, 1988b). Figure 2 reports results obtained in our laboratory in obese and lean Zucker rats fed fish oil in the diet at very low concentration for 12 weeks. The  $\Delta 6$ -desat-

uration rate was measured in liver microsomes with LA and LNA as substrates. The  $\Delta 5$ -desaturation rate was measured with DGLA as substrate. The results illustrated in figure 2 confirm what was previously demonstrated (Blond *et al*, 1989) that  $\Delta 6$ - and  $\Delta 5$ -desaturase activities are lower in obese rats than in control lean rats, especially with the n-6 fatty acids as substrates. Additionally, the fish oil diet highly depressed the  $\Delta 6$ -desaturase activity but uniquely with LA as a substrate and to a higher extent in lean rats. The same was true with  $\Delta 5$ -desaturation of DGLA to AA. The depressed capacity of liver microsomes to synthesize AA from LA was reflected in the AA content of microsomal phospholipids which was decreased, while the DHA content was increased. Surprisingly in these experiments the AA content was not decreased in heart phospholipids of rats fed the fish oil diet. It was even increased in kidney phospholipids of obese rats, but not of lean rats. Since heart and kidney exhibit low capacity of desaturation, the unaltered fatty acid profile of the phospholipids of these organs could result from a preferential uptake of the long-chain EFA from the blood lipids.

Dietary n-6 fatty acids also alter desaturation rates. Periago *et al* (1989) observed in rats that feeding corn oil, containing nearly 60% 18:2n-6, increased the  $\Delta 6$ -desaturation rate by more than 2 fold when compared to olive oil containing 72% 18:1n-9. With sunflower oil (32% 18:2n-6) in the diet, Christiansen *et al* (1991) observed the same extent of increase of  $\Delta 6$ -desaturation rate. In addition, the authors showed a 2.5-fold increase in  $\Delta 5$ -desaturation rate. These results are very similar to what was observed with dietary LNA.

GLA (18:3n-6), the product of  $\Delta 6$ -desaturation of LA (18:2n-6), is present in evening primrose, borage and blackcurrant seed oils. When added to the diet, they modify  $\Delta 6$ - and  $\Delta 5$ -desaturation rates, the effect depending on age, strain of rats and type



**Fig 2.**  $\Delta 6$ - and  $\Delta 5$ -desaturation of n-6 and n-3 essential fatty acids in liver microsomes from obese and lean Zucker rats fed a fish-oil diet (FO) compared to control (CO).  $\square$  Control rats (CO);  $\square$  fish-oil fed rats (FO); \*  $P < 0.05$ : FO versus CO in each phenotype; a, b  $P < 0.05$ : obese versus lean rats fed the control diet.

of GLA supplementation. Høy *et al* (1983) observed that the activities were not affected by dietary GLA. However, Choi and Sugano (1988) found a decrease in  $\Delta 6$ -desaturase activity in 8-month-old but not in 3-week-old rats. On the other hand, Biagi *et al* (1991) showed an increase in  $\Delta 6$ -desaturation rate of LA and particularly of LNA. In recent experiments, we observed age-dependent variations in  $\Delta 6$ -desaturation of LA and LNA and in  $\Delta 5$ -desaturation of DGLA in liver from Wistar rats fed GLA (unpublished results). In obese Zucker rats, dietary GLA bypasses the  $\Delta 6$ -desaturase and tends to correct the low AA content of tissues (Girault *et al*, 1992).

Very few experiments have dealt with dietary AA (20:4n-6). In the same set of experiments as above on Zucker rats, we observed very similar effects as with the n-3 long-chain fatty acids EPA + DHA (fig 2), namely a marked decrease in  $\Delta 6$ -desaturation rate of LA, no effect on  $\Delta 6$ -desaturation rate of LNA and a decrease in  $\Delta 5$ -desaturation of DGLA but less pronounced than  $\Delta 6$ -desaturation of LA (unpublished results).

In summary, with regard to the n-6 and n-3 EFA, the precursors 18:2n-6 and 18:3n-3 highly stimulate the desaturases involved in the biosynthesis of longer chain PUFA (20:4n-6, 20:5n-3, 22:6n-3), while these acids present in the diet markedly decrease  $\Delta 6$ -desaturation of 18:2n-6, less markedly  $\Delta 5$ -desaturation of 20:3n-6, but do not alter  $\Delta 6$ -desaturation of 18:3n-3. This explains why biosynthesis of 20:4n-6 was decreased by dietary fish oil. However, dietary 20:4n-6 also decreased 22:6n-3, in phospholipids according to our results. As stated by Zevenberger and Houtsmuller (1989), the  $\Delta 6$ -desaturase system alone does not totally determine the PUFA levels in tissue lipids. Other factors, such as the incorporation rate of PUFA in tissue phospholipids are also involved.

Other fatty acids are likely to modify desaturation rates. Oleic acid (18:1n-9) can also inhibit  $\Delta 6$ -desaturase activity but high intake levels are required (Holman, 1964). Data from Periago *et al* (1989) also indicate that dietary 18:2n-6, compared to 18:1n-9, was much more efficient in stimulating  $\Delta 6$ -desaturation rate.

Trans-fatty acids have been reported to inhibit the conversion of LA to AA, especially the  $\Delta 6$ -desaturation step, as pointed out in previous reviews (Kinsella *et al*, 1981; Emken, 1984). This effect could be due to essential fatty-acid deficiency. On the contrary,  $\Delta 5$ -desaturation was either increased (Blomstrand *et al*, 1985) or not affected (De Gomez Dumm *et al*, 1983; Mahfouz *et al*, 1984). Results by Blond *et al* (1990) showed that dietary heated linseed oil containing 18:3n-3 trans-isomers did not modify liver capacity to desaturate 18:2n-6 and increased  $\Delta 5$ -desaturation activity. In cultured human skin fibroblasts, trans-fatty acids (elaidate and linoelaidate) are potent inhibitors of  $\Delta 6$ -desaturation (Rosenthal and Whitehurst, 1983) and  $\Delta 5$ -desaturation (Rosenthal and Doloresco, 1984).

Cyclopropenoic fatty acids, which possess a cyclopropene ring in the acyl chain, are present in certain oils, such as baobab seed oil. They are potent inhibitors of  $\Delta 6$ - and  $\Delta 5$ -desaturation both *in vitro* and *in vivo* (Cao *et al*, 1993). They induced a marked decrease in AA content of tissue phospholipids in rats.

The mechanism by which dietary fatty acids modify the desaturase activity may vary according to the fatty acids. The  $\Delta 6$ -desaturase is sensitive to feed-back inhibition by the products of desaturation and elongation (Brenner, 1981), which is consistent with its high depression by long-chain n-6 and n-3 polyunsaturated fatty acids. Leikin and Brenner (1989) have reported that a cytosolic factor prevented product inhibition of both  $\Delta 6$ - and  $\Delta 5$ -desaturation by GLA and AA, respectively, as they are formed. Hence, the presence or absence of this factor could regulate the flow of products through this pathway.

These mechanisms intervene in short-term regulation, or *in vitro*. However, *in vivo*, additional mechanisms should be implicated, such as regulation of the amount of desaturase in microsomes. In a recent study,

Osmundsen and Christiansen (personal communication) demonstrated that fish oil diet (20% wt) led to 90% decrease in  $\Delta 9$ -desaturase activity while the decrease in hepatic  $\Delta 9$ -desaturase mRNA was 60%. This demonstrated that the n-3 polyunsaturated fatty acids in fish oil regulate gene expression of  $\Delta 9$ -desaturase in rat liver. However, since the desaturase activity was less decreased than the desaturase mRNA, other types of regulation must take place such as lower translation rate of mRNA to protein or lower stability of mRNA. Similar results were obtained with soybean oil containing both 18:2n-6 and 18:3n-3 but were less pronounced. If probes for  $\Delta 6$ - and  $\Delta 5$ -desaturases were available, this type of study could be undertaken to assess whether or not n-6 and n-3 PUFA are also potent inhibitors of gene expression for  $\Delta 6$ - and  $\Delta 5$ -desaturases. The regulation of hepatic gene expression by PUFA has been well documented for enzymes involved in lipogenesis (Clark and Jump, 1993).

Modification by dietary lipids of the phospholipid fatty acid profile of membranes in which the desaturase systems are anchored are also likely to modify the expression of desaturation activity through changes in fluidity.

Nutrients other than fatty acids modulate desaturase activities. Garda and Brenner (1985) reported that, *in vitro*, the incorporation of cholesterol into rat liver microsomes enhanced the  $\Delta 6$ - and  $\Delta 5$ -desaturase activity, probably by decreasing membrane fluidity. On the contrary, *in vivo*, cholesterol incorporated into diet decreased the  $\Delta 6$ - and  $\Delta 5$ -desaturase activity by a mechanism yet to be elucidated (Leikin and Brenner, 1987; Garg *et al*, 1988a,b).

Dietary proteins that are important components of diet also modulate desaturase activities. A low dietary intake of proteins evokes, in growing rats, a marked decrease in  $\Delta 6$ - and  $\Delta 5$ -desaturation rate in liver microsomes (Narce *et al*, 1988). After bal-

anced refeeding, the desaturase activities are rapidly recovered. High protein intake, *ie* above 35% of the calories consumed, increases  $\Delta 6$ -desaturase activity (Brenner, 1989). Ingested ethanol was also observed to decrease  $\Delta 6$ - and  $\Delta 5$ -desaturase activities (Nervi *et al*, 1980; Wang and Reitz, 1983).

Data concerning the effect of dietary oligonutrients, such as vitamins and minerals, are very scarce, despite their importance in animal health. Zinc deficiency caused increase in  $\Delta 6$ -desaturase activity in mammary tissue (Cunnane and Wahle, 1981) while it had no effect on  $\Delta 6$  desaturation in liver and decreased the rate of  $\Delta 5$  desaturation (Cunnane, 1988). High sodium chloride intake influences  $\Delta 6$ - and  $\Delta 5$ -desaturation rate in rat liver microsomes. When 10-week-old male Wistar rats received 3% NaCl in drinking water for 60 d, the  $\Delta 6$ -desaturation of LA and  $\Delta 5$ -desaturation of DGLA in liver microsomes were enhanced by 50 and 75% respectively (Narce *et al*, 1992). On the contrary, in another set of experiments in 4-week-old genetically normotensive rats (WKY = Wistar-Kyoto) receiving 1% NaCl in drinking water for 4 weeks, the  $\Delta 6$ - and  $\Delta 5$ -desaturation rates in liver microsomes were decreased by 25 and 28% respectively (Poisson *et al*, 1993b). The observed opposite effects could be due to different experimental conditions: strain and age of rats, amount and duration of salt intake, in particular. The effect evoked by this nutritional situation may involve modifications of steroid secretion (aldosterone) by adrenals.

Another example of relation between a nutritional state and the animal hormonal status is food deprivation. The effects of fasting are probably related to increased glucagon secretion, whereas the effects of refeeding could be related to increased insulin secretion by endocrine pancreas. The effects of fasting have been recently reviewed (Poisson and Cunnane, 1991). Several studies have shown that food deprivation

in rats decreased  $\Delta 6$ -desaturation of LA. In rats fasting for at least 12 h, compared to control fed rats, the desaturation of LA was only half or less of control (Brenner, 1968; De Gomez Dumm *et al*, 1970; Brenner *et al*, 1974). Refeeding fasting animals for only 12 h with 10% glucose in the drinking water, largely restored the activity of  $\Delta 6$ -desaturation, but the increase was transient (De Gomez Dumm *et al*, 1970). The  $\Delta 6$ -desaturation of LNA was also decreased in fasting rats while refeeding restored the activity (Brenner, 1974; Nervi *et al*, 1975). In contrast to  $\Delta 6$ -desaturation,  $\Delta 5$ -desaturation rate of DGLA in liver microsomes was increased after 12 h of fasting in rats previously fed a balanced diet (Lee and Sprecher, 1971). This increase was maintained for 48 h and then declined to control values after 72 h of fasting.

### **Hormonal factors**

Many dietary modifications influence the hormonal status of individuals and the effects of dietary factors on the liver capacity to synthesizing long-chain PUFA from the precursors LA and LNA might be often explained by action of the hormones of which secretion is enhanced or inhibited. Insulin and glucagon are particularly concerned since they are involved in the regulation of energy metabolism in fed and fasting state, respectively. The severe metabolic disturbances observed in the human type I diabetes that is insulin-dependent, underlines the importance of this hormone. Moreover, studies on lipid metabolism in diabetes have provided a wealth of information on changes in essential fatty acid metabolism (Poisson and Cunnane, 1991).

Mercuri *et al* (1966), Brenner *et al* (1968), Lyman (1971) and Brenner (1989) have shown that the desaturation of LA to GLA was severely depressed in liver microsomes from alloxan-diabetic rats to reach only

22–56% of the control values. Treatment of diabetic animals with insulin corrected this defect in part or totally (table IV). Friedmann *et al* (1966) also demonstrated *in vivo* that the synthesis of AA from LA was decreased in alloxan-diabetic rats. Several authors later confirmed that insulin restored to control the  $\Delta 6$ -desaturation rate of LA and this apparently by enzyme induction, since inhibition of protein synthesis impaired the recovery of enzyme activity (Brenner, 1989). The  $\Delta 6$ -desaturation of LNA is also decreased in alloxan-diabetes, but to a lesser extent (Mercuri *et al*, 1967). The effect is corrected by insulin but after a longer time than for  $\Delta 6$ -desaturation of LA (Eck *et al*, 1979). Poisson *et al* (1978) have demonstrated that  $\Delta 5$ -desaturation of DGLA into AA was also depressed in alloxan-induced diabetic rats *in vivo*. This effect was demonstrated to be reversed with subcutaneous injection of insulin to streptozotocin-diabetic rats, providing evidence of insulin regulation of  $\Delta 5$ -desaturation as a secondary determinant of LA metabolism to AA. The defect *in vivo* of  $\Delta 5$ -desaturation of DGLA and its correc-

tion by insulin, have also been shown in rat liver homogenates *in vitro* (Friedmann *et al*, 1966).

In the genetically diabetic rat (BB),  $\Delta 6$ - and  $\Delta 5$ -desaturation are similarly defective and are restored by insulin injection (Mimouni and Poisson, 1990). As shown in figure 3, when BB rats were equilibrated by insulin injection (3 h), the  $\Delta 6$ - and  $\Delta 5$ -desaturation rates were similar to values in control Wistar rats. When insulin had decreased in blood (17 and 48 h), the desaturation rates had considerably decreased, especially the  $\Delta 5$ -desaturation of DGLA. Elongation of DGLA was also demonstrated to be insulin dependent (Mimouni *et al*, 1992). Very recently, Mimouni *et al* (1994) have examined the  $\Delta 4$  (n-6) desaturation in the same strain of rats and shown that the pattern of responses was similar to that of  $\Delta 6$ - and  $\Delta 5$ - (n-6) desaturations with a non-parallel relationship between the desaturation system and the glycemia.

In humans, El Boustani *et al* (1989) have measured the desaturation of deuterated DGLA into AA in plasma lipids of newly diagnosed diabetics who had never received insulin. After insulin treatment they found that the concentration of deuterated AA increased significantly from pretreatment values, suggesting that insulin was involved in the activity of  $\Delta 5$ -desaturation in humans. Other studies have similarly suggested that desaturation of LA was sensitive to insulin status (Poisson and Cunnane, 1991).

Glucagon and epinephrine are antagonistic to insulin and depress  $\Delta 6$ - and  $\Delta 5$ -desaturase activity in rat liver microsomal preparations (De Gomez Dumm *et al*, 1975, 1976). Glucagon also prevents the refeeding-induced activation of  $\Delta 6$ -desaturation in previously fasted rats. Depression of  $\Delta 6$ -desaturation by glucagon and epinephrine is thought to be mediated by stimulation of adenylyl cyclase and the subsequent increase in intracellular cyclic AMP, because dibutyryl cyclic AMP induces the same effect as

**Table IV.** Hormonal factors affecting  $\Delta 6$ - and  $\Delta 5$ -desaturation in rat liver microsomes.

	$\Delta 6$	$\Delta 5$
Diabetes	-	-
Diabetes + insulin	+	+
Glucagon	-	-
Epinephrine	-	-
ACTH	-	-
Glucocorticoids	-	-
Hypothyroidism	-	-
Hypothyroidism + thyroxine	+	+
Hyperthyroidism	-	-
Deoxycorticosterone or aldosterone	-	-
Oestradiol (in ovariectomized females)	-	-

+ stimulation; - inhibition; references in the text.



glucagon (Brenner, 1977). Glucagon and cAMP also inhibit the phosphorylation of acetyl CoA carboxylase, thereby inhibiting formation of malonyl-CoA. This may decrease the elongation rate of 18:3n-6, the product of  $\Delta 6$ -desaturation of 18:3n-6. The resulting increased concentration of 18:3n-6 may reduce the activity of  $\Delta 6$ -desaturation by feedback inhibition (Brenner and Peluffo, 1969).

Similarly to glucagon and epinephrine, the glucocorticoids also affect the metabolism of glucose and fatty acids. They also inhibit  $\Delta 6$ -desaturase activity (Brenner, 1981). However, they require more time than epinephrine to inhibit desaturation. The delay was attributed to suppression of protein synthesis. Pituitary adrenal corticotrophic hormone (ACTH) causes a decrease in  $\Delta 6$ - and  $\Delta 5$ -desaturation activity in rat liver and adrenal glands (Mandon *et al*, 1986). Since ACTH stimulates glucocorticoid secretion, the effect of the hormone may be direct or/and indirect.

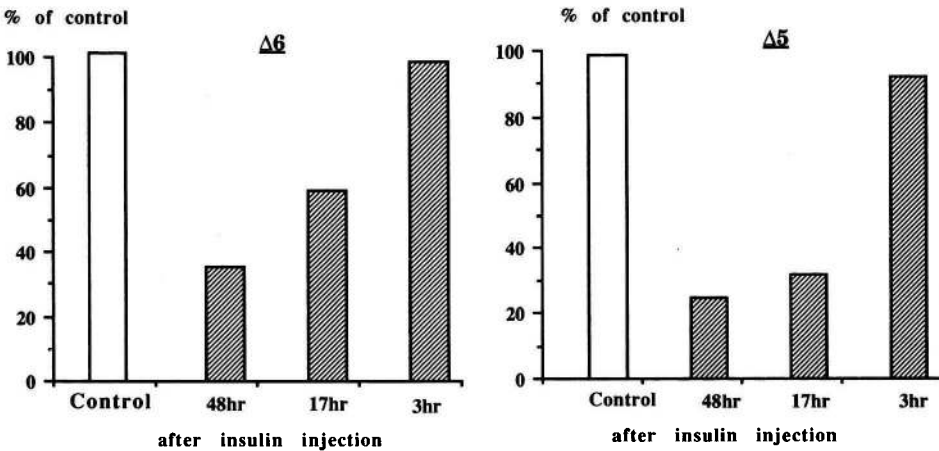
ACTH and glucocorticoids, the secretion of which has a circadian rhythm, may be

involved in the circadian rhythm of desaturase activities, being lowest around 8 am in normal day-night cycle (Brenner, 1981).

Thyroid hormones are necessary for  $\Delta 6$ -desaturation since hypothyroidism greatly decreased the  $\Delta 6$ -desaturation rate of LA in rat liver microsomes (Faas and Carter, 1982). While treatment of hypothyroid rat restores this activity to normal values, hyperthyroidism is associated with a decrease in  $\Delta 6$ -desaturation (Hoch, 1981; Faas and Carter, 1982). The lower  $\Delta 6$ -desaturation rate in liver microsomes from obese Zucker rats, compared to their lean littermates, were also ascribed by Blond *et al* (1989) to hypothyroidism in these animals.

The levels of thyroxine, glucocorticoids, epinephrine and glucagon increase during fasting (Brenner, 1981) and alteration of  $\Delta 6$ -desaturase activity in fasted rats is probably modulated in part by these metabolic hormones.

Hormones involved in sodium metabolism in kidneys are also active in inhibiting desaturation in rat liver microsomes (De Alaniz and Marra, 1992). These authors observed that when liver micro-



**Fig 3.**  $\Delta 6$ - and  $\Delta 5$ -desaturation of n-6 essential fatty acids in liver microsomes from female genetically diabetic (BB) rats 3, 17 and 48 h after they have received a subcutaneous injection of insulin compared to control Wistar rats. Values at 17 and 48 h were not significantly different ( $p < 0.05$ ). They were different from values at 3 h. Those were not significantly different from control values. Reproduced from Mimouni and Poisson (1990).

somes from deoxycorticosterone- or aldosterone-treated rats were incubated with DGLA, the  $\Delta 5$ -desaturation rate was considerably reduced, to 38 and 32% of the control values respectively. High sodium intake decreases aldosterone secretion. This could in part explain the increased  $\Delta 6$ -desaturation rate observed by Narce *et al* (1992) in microsomes from rats drinking salted water.

Oestradiol, injected to ovariectomized female rats, decreases the  $\Delta 6$ -desaturase activity in liver microsomes (Gonzales *et al*, 1986).

In conclusion, as could be expected, hormones greatly influence desaturation rates in liver and by this means may modify the biosynthesis of long-chain EFA and their availability in tissues. The subsequent modification of fatty-acid profile of membrane phospholipids or the modified availability of substrates for eicosanoid synthesis, could mediate certain properties of hormones and also modify the response of cells to hormone stimulation.

## DEVELOPMENT AND AGING

Regulation of long-chain EFA synthesis in the different stages of life in animals is crucial, since at early ages, the requirement by developing tissues, such as the brain, is elevated, whereas, in aging, different pathologies occur which can be prevented or retarded by suitable dietary intake of EFA (Kinsella, 1991).

The developing brain and central nervous system contain substantial amounts of lipids, both in white matter and myelin (50–70%) and in grey matter consisting primarily of neuronal cells (nearly 40%) (Purvis *et al*, 1982), that is appreciably more than in most body organs, including liver, kidney and heart. Total phospholipids of brain also have distinctive fatty acyl chain composi-

tion, since the ratio of n-6 to n-3 fatty acids is close to 1:1 compared to 4:1 in liver, kidney and heart (Bourre *et al*, 1989). The 20:4n-6 is the prominent member of the n-6 family, accounting for 10–15% of total fatty acyl chains while, in the n-3 family 22:6n-3 predominates at 6–15% of the total. To achieve this tissue-specific composition during normal brain growth and development requires an appropriate supply of PUFA, both in quantity and in balance between the n-6 and n-3 fatty acids.

Requirements for PUFA are particularly critical for the developing fetus, because of its rapid growth and the early development of its brain. PUFA of the n-6 and n-3 series, or their precursors LA and LNA, are provided from the mother's blood. When the precursors are provided, they have to be converted into PUFA by the fetus. In rats, liver microsomes were demonstrated to be capable of transforming 18:2n-6 into 18:3n-6 by  $\Delta 6$ -desaturation (Satomi and Matsuda, 1973; Pascaud and Strouve-Vallet, 1985; Ravel *et al*, 1985). They were also capable of transforming 20:3n-6 into 20:4n-6 by  $\Delta 5$ -desaturation (Ravel *et al*, 1985). The  $\Delta 6$ -desaturation of 18:2n-6 was also detected in fetus brain microsomes in rats (Pascaud and Strouve-Vallet, 1985) and in mouse brain homogenates (Bourre *et al*, 1990). Recent *in vivo* experiments (Green and Yavin, 1993) in which labelled 18:3n-3 and 18:2n-6 were intracranially injected into near-term fetal rats, clearly showed that these acids were converted to long-chain polyenoic derivatives in the fetal brain. Therefore, the fetus can, at least in part, supply its own n-6 and n-3 PUFA requirements. However, especially at the early steps of development, it also relies on its mother's supply.

In animals, the liver is the principal organ capable of synthesizing PUFA from the LA and LNA precursors and to secrete these long-chain fatty acids into the blood. However, in pregnant females, placenta can also

be a source of PUFA in certain species. While no activity of  $\Delta 6$ - and  $\Delta 5$ -desaturations was detected in rat placenta microsomes (Ravel *et al*, 1985), the ovine placenta was shown to desaturate 18:2n-6 into trienoic and tetraenoic fatty acids (Shand and Noble, 1981). The existence of desaturation activity in human placenta is still controversial. Certain authors detected some biosynthesis activity (Zimmermann *et al*, 1979), while others did not (Chambaz *et al*, 1985).

Therefore, the developing fetus must depend largely on placental transfer of maternal long-chain EFA, particularly for n-3 fatty acids, highly present in neural tissues. This underlines the importance of EFA intake and of the liver desaturation capacity in pregnant women.

After birth, the neonate must also principally rely on mother's milk for supply the long-chain fatty acids for tissue growth (Martin *et al*, 1993), although  $\Delta 6$ - and  $\Delta 5$ -desaturation activities are present in neonates aged 2–14 d (Poisson *et al*, 1993a), as they are in human adults (Blond *et al*, 1981).

Because of its importance, the brain deserves particular alteration. What is the capacity of the developing brain for conversion of extracellular supplies of 18:3n-3 and 18:2n-6 into essential products such as 20:5n-3, 22:6n-3 and 20:4n-6? To what extent does the brain depend on the liver for supplying the essential PUFA? These are important questions regarding the EFA availability in neonates.

*In vitro* preparation from brain and liver (Cook, 1978) showed that in the very young 10-day-old rats, conversion of 18:2n-6 and 18:3n-3 by  $\Delta 6$ -desaturation was much higher in brain than in liver. The  $\Delta 5$ -desaturation of 20:3n-6, although very low in brain, was also significantly higher than in liver. Bourre *et al* (1983) have demonstrated that mouse brain cells were not capable of synthesizing 22:6n-3 from 18:3n-3 but were capable of taking it up from the medium.

The brain 22:6n-3 would thus have a hepatic origin, being specifically taken up by endothelial cells of brain from the blood lipids (Bourre *et al*, 1983; Scott and Bazan, 1989). Indeed, as shown by Anderson and Connor (1988), rat brain exhibits a specific capacity *in vivo* to take up 22:6n-3, although less than liver.

On the other hand, Cook (1991) thinks that the brain is capable of synthesizing 22:6n-3. Indeed Spector and Moore (1992) by using cultured endothelial cells from brain vessels, cultured astrocytes, and cultured neurons from rats, clearly showed that endothelial cells and astrocytes actively cooperated in synthesizing 22:6n-3 and providing it to neurons. Endothelial cells extensively convert 18:3n-3 to 20:5n-3 while astrocytes convert the latter into 22:6n-3 available for neurons. Synthesis of 20:4n-6 from 18:2n-6 would follow the same pathway.

As animals are growing older, the capacity of liver to desaturate n-6 and n-3 fatty acids increases while that of brain decreases (Cook, 1991). However, at high ages desaturation *in vitro* of fatty acids in rat liver (Peluffo and Brenner, 1974; Bordini *et al*, 1988; Choi *et al*, 1989; Biagi *et al*, 1991) and in mouse liver (Bourre *et al*, 1990) was shown to decrease. In a recent study (Mansiongui *et al*, 1993), we have measured  $\Delta 6$ -desaturation of 18:2n-6 and 18:3n-3 and  $\Delta 5$ -desaturation of 20:3n-6 in liver microsomes from rats fed a balanced diet throughout their life from 1.5 to 24 months of age. The results were expressed as specific activity and as liver capacity to desaturate by taking into account the liver weight which increased from 6.8 g at 1.5 month to 14.1 g at 24 months. The results illustrated in figure 4 show that specific activity of 18:2n-6 had not decreased from 1.5 to 24 months of age, despite some variation throughout the life. Since the liver weight increased, the liver capacity to desaturate 18:2n-6 increased up to 6 months to reach a plateau from 6 to 24 months. The time-

course of  $\Delta 6$ -desaturation of 18:3n-3 was different from that of 18:2n-6, since it increased from 1.5 to 3 months and then regularly decreased. However, the liver capacity to desaturate 18:3n-3 was not different at 1.5 and 24 months. The  $\Delta 5$ -desaturation of 20:3n-6 varied with age very differently from  $\Delta 6$ -desaturation of 18:2n-6. It was nearly 6-fold higher at 1.5 months and fell to nearly the same value at 3 months. From then on,  $\Delta 5$ -desaturation slowly increased up to 24 months. At this age, the liver capacity to desaturate 20:3n-6 was not significantly different from that measured at 1.5 months.

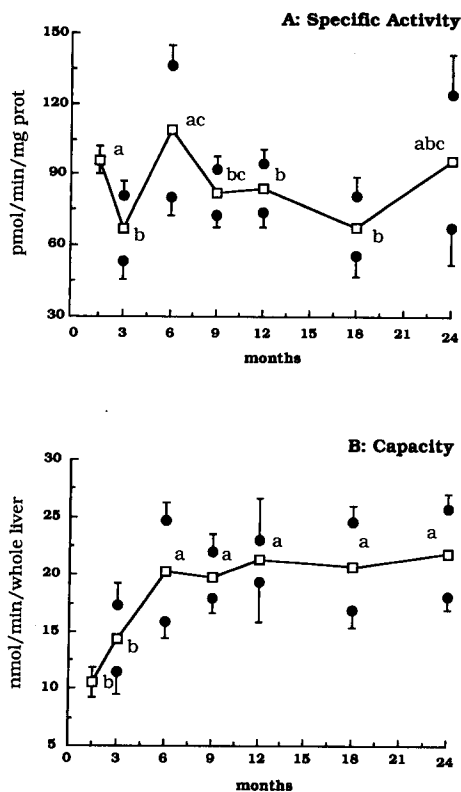
These data demonstrated that the liver capacity to synthesize 20:4n-6 from 18:2n-6 did not decrease with age in rats. This was reflected in 20:4n-6 content of liver microsomal phospholipids (fig 5) and of erythrocyte lipids, which increased from 1.5 to 20 months. In these experiments, the liver capacity to convert 20:4n-3 to 20:5n-3 by  $\Delta 5$ -desaturation was not determined, because commercial labelled precursor was not available. The liver capacity to desaturate 18:3n-3 was not decreased at 24 months, and the liver microsomal phospholipid content in 22:6n-3 was not significantly different at 1.5 and 24 months (12.9 and 12.5 mol% respectively).

These results clearly show that in rats fed a balanced diet, if desaturation capacity of liver is evaluated in the whole organ, no decrease occurs from 1.5 to 24 months. This is reflected in the unchanged or even increased content in polyunsaturated n-6 and n-3 fatty acids of the membrane lipids studied.

In humans, the liver desaturation activities are much lower than in rats (De Gomez Dumm and Brenner, 1975; Blond *et al*, 1981). Despite that, several studies have shown that few differences exist between elderly and young subjects with respect to the n-6 and n-3 fatty acid content of serum (Holman *et al*, 1979; Asciti-Moura *et al*,

1988), erythrocyte (Driss *et al*, 1988) and platelet (Vericel *et al*, 1988) phospholipids.

It can thus be assumed that in humans, as in rats, the desaturation activities are regulated to maintain constant PUFA levels in membrane phospholipids.



**Fig 4.** Age-related changes in  $\Delta 6$ -desaturation of linoleic acid (18:2n-6) in liver microsomes expressed both as specific activity of liver microsomes (A) and activity of whole liver or capacity (B). Results are means  $\pm$  SD obtained from 1 group of 6 rats (1.5 months) or 2 groups of 3 rats at all other ages. After analysis of variance using the Fisher multiple range test, means of the 6 rats for each age were compared according to the least significant difference and classified in decreasing order. Means assigned different superscript letters were significantly different ( $P < 0.05$ ). Prot: protein. Reproduced from Maniongui *et al* (1993).

## OXIDATION OF ESSENTIAL FATTY ACIDS

When entering the animal cells, EFA compete for several metabolic pathways in different compartments of the cell, generally anabolic pathways (microsomal esterification) and catabolic pathways (mitochondrial and peroxisomal  $\beta$ -oxidation). Generally, especially in the liver, regulation of oxidation may modulate availability of fatty acids for esterification. However, the regulation of the activity of other enzymes such as acyltransferases can also direct acids to synthesis of phospholipids, cholesteryl esters or even triacylglycerols.

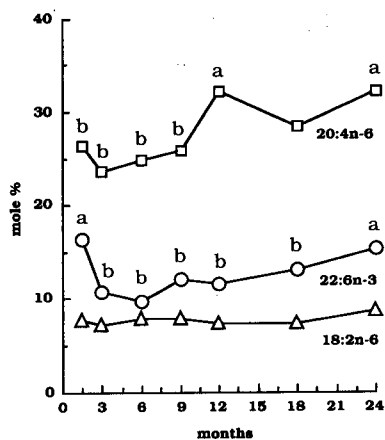
The overall oxidation of fatty acids in the body results in  $\text{CO}_2$  production depending on fatty-acid structure. Leyton *et al* (1987) have investigated the difference in rates of whole-body oxidation of essential PUFA in the rat. Using radiolabelled substrates, the oxidation rate of oleic (18:1n-9), linolenic

(18:2n-6),  $\alpha$ -linolenic (18:3n-3),  $\gamma$ -linolenic (18:3n-6), dihomo- $\gamma$ -linolenic acid (20:3n-6) and arachidonic (20:4n-6) acids was determined by measuring the extent of label expired in  $\text{CO}_2$  over a 24 h period. Labelled fatty acids were administered orally dissolved in olive oil. The results, illustrated in figure 6, are expressed as percentage of radioactivity administered. Seven hours after oral administration of fatty acid, nearly 50% of the radioactivity supported by 18:1n-9 was recovered as  $^{14}\text{CO}_2$ . For the other unsaturated fatty acids, the amount of recovered radioactivity decreased in the order: 18:1n-9 (49%) > 18:3n-3 (41%) > 18:2n-6 (27%) > 18:3n-6 (13%) > 20:3n-6 = 20:4n-6 (4%). The order was the same after 24 h except that 18:3n-3 was recovered to a greater extent than 18:2n-6.

From these data, it is evident that long-chain essential n-6 fatty acids were oxidized to  $\text{CO}_2$  at a very low rate, much lower than the 'parents' 18:2n-6 and 18:3n-3. This is evidence that when provided as very long-chain fatty acids, the EFA are required in lower amount in the diet than the precursors 18:2n-6 and 18:3n-3.

The same type of experiment has been carried out in humans using [ $^{13}\text{C}$ ]-labelled fatty acids (Jones *et al*, 1985). [ $^{13}\text{C}$ ]Oleic, [ $^{13}\text{C}$ ]linoleic or [ $^{13}\text{C}$ ]stearic acid was given in a capsule together with the breakfast meal to male adult volunteers. Subjects were confined to a metabolic ward and provided 6 min breath samples hourly during a 10 h period, for  $^{13}\text{CO}_2$  enrichment analysis by mass spectroscopy. Results showed that the oxidation rate was in the order: 18:1n-9 > 18:2n-6 > 18:0.

The maximum of  $^{13}\text{CO}_2$  excretion was observed 6 h after oral administration for the first 2 fatty acids. It was delayed for 18:0. During the 6th hour,  $^{13}\text{C}$  recovered by oxidation of 18:1n-9 was around 2.8% of the absorbed dose. It was 2.0% for 18:2n-6 and 0.1% for 18:0, that is 28-fold less than for 18:1n-9.

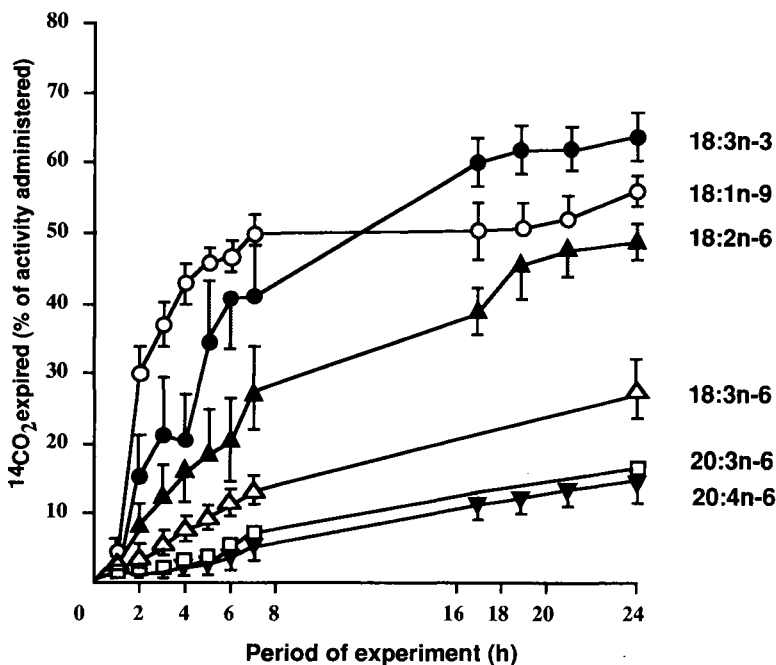


**Fig 5.** Age-related changes in percentages of major polyunsaturated fatty acids in liver microsomal phospholipids. Results are means from 6 rats at each age. After analysis of variance using the Fisher multiple range test, the means were compared according to the least significant difference and classified in decreasing order. Means assigned different superscript letters were significantly different ( $p < 0.05$ ). Reproduced from Maniongui *et al* (1993).

As in rats (Leyton *et al*, 1987), 18:2n-6 in humans is oxidized at lower rate than 18:1n-9. Oxidation by tissues thus limits the availability of the absorbed LA. The same is probably true, in humans, for LNA as well.

In the whole body, the oxidation rate of fatty acids by tissues depends on the rate of their uptake by the cells and the rate of their oxidation inside the cells. The uptake rate of fatty-acids from the blood depends itself on tissue or organ specificity, on fatty-acid specificity and on whether or not the fatty acid is esterified. Although the EFA are principally present in the blood in an esterified form, especially following their intestinal absorption (Carrier *et al*, 1991), most of the experiments undertaken for studying differential uptake by tissues have utilized free

fatty acids. In that case, the data reported above and those obtained by Anderson and Connor (1988) have clearly shown that, per unit of wet tissue, the liver took up fatty acids in greater amounts than the brain, 13–38 times more depending on the fatty acid, although the rats were very young and the need for fatty acids of the developing brain was very high. Apparently, other organs and tissues in the rat take up fatty acids less rapidly than liver (Ong *et al*, 1977). However, because of their weight in animals, skeletal muscles and adipose tissue play a major role in uptake (adipose tissue) and oxidation (muscles) of fatty acids. But liver occupies a key position because, like intestine, it possesses the ability to provide tissues with secreted fatty acids. Whether or



**Fig 6.** The *in vivo* oxidation of unsaturated fatty acids in rats a 24-h period. Each animal received an oral dose of 5–6  $\mu\text{Ci}$   $^{14}\text{C}$ -labelled fatty acid in 0.2 ml olive oil and was put into a metabolism chamber and the expired  $^{14}\text{CO}_2$  was collected. Points are mean values, with their standard errors represented by vertical bars, for the radioactivity recovered in  $\text{CO}_2$  from ( $^{14}\text{C}$ )  $\alpha$ -linolenic acid (●); ( $^{14}\text{C}$ ) oleic acid (○), ( $^{14}\text{C}$ ) linoleic acid (▲), ( $^{14}\text{C}$ )  $\gamma$ -linolenic acid (△), ( $^{14}\text{C}$ ) dihomo- $\gamma$ -linolenic acid (□) and ( $^{14}\text{C}$ ) arachidonic acid (▼). Reproduced from Leyton *et al* (1987).

not fatty acids are taken up by the liver, more or less rapidly according to acyl-chain specificity is still controversial. Christensen *et al* (1986) observed that, at a given free fatty acid/serum albumin molar ratio, the uptake of unsaturated fatty acids was identical whatever the fatty acids. This confirmed results by Kohout *et al* (1971) in perfused liver for saturated and unsaturated fatty acids. On the contrary, Anderson and Connor (1988) have shown that, *in vivo*, liver of very young rats took up 22:6n-3 4 times more rapidly than 18:3n-3.

The mechanism of fatty-acid uptake by animal cells has not been fully elucidated. In spite of strong evidence for carrier-mediated uptake and possibly for active transport, it is still argued that fatty-acid uptake occurs by spontaneous and non-specific diffusion of fatty acids across the plasma membrane (Schulz, 1991).

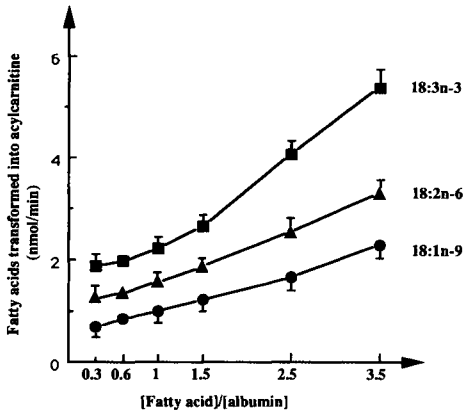
Inside the cell, fatty acids are channelled to different metabolic pathways, possibly by fatty-acid binding proteins (FABP) (Schulz, 1991). In most cells, particularly in hepatocytes, the 2 major routes are esterification in endoplasmic reticulum and oxidation in mitochondria and peroxisomes. In liver, regulation of oxidation of fatty acids generally determines their availability for esterification. However, the esterification pathway is also regulated. Fish oil diets that greatly increase mitochondrial and peroxisomal  $\beta$ -oxidation of fatty acids, conversely decrease phosphatidate hydrolysis and thence glycerolipid synthesis (Halminski *et al*, 1991). The tissues and organs in animals display different oxidative capacities. In experiments with homogenates of liver, heart muscle and quadriceps muscle, Reubsæet *et al* (1989) have observed that the fatty-acid oxidation rate in the heart was 52 to 93% that of the liver, whereas the rate in the skeletal muscle was only 9 to 35% of the liver capacity.

The contribution of mitochondria and peroxisomes to fatty-acid oxidation highly depends on the fatty acid (Osmundsen *et*

*al*, 1991). In their study on tissue homogenates, Reubsæet *et al* (1989) have observed that the contribution of peroxisomal fatty acid oxidation to overall tissue oxidation was, depending on the fatty acid, 15 to 28% in liver, 14 to 46% in heart and 18 to 38% in the quadriceps muscle. The difference is assumed to be the mitochondrial oxidation. In liver homogenate, 20:4n-6, 22:4n-6 and 18:1n-9 were preferentially (> 80%) oxidized by mitochondria. 18:2n-6 was also principally oxidized by mitochondria but to a lesser extent (72%). Some differences between tissues were demonstrated in this study. In a similar experiment in isolated liver cells, Christensen *et al* (1986) observed that 22:4n-6 and 20:5n-3 were oxidized to a greater extent in peroxisomes than 18:1n-9, 18:2n-6, 20:4n-6 and 22:6n-3. In this experiment, 75% of 22:4n-6 oxidation was in peroxisomes. The difference between the 2 experiments with regard to the location of 22:4n-6 oxidation may be due to the model used.

Osmundsen *et al* (1987) have measured fatty-acid oxidation on isolated mitochondria and peroxisomes. They observed that in mitochondria, 18:1n-9, 18:2n-6, and 18:3n-3 were oxidized at nearly equal rates, while 18:3n-6, 20:4n-6 and 22:6n-3 were poorly oxidized. In peroxisomes, 18:3n-3, 18:3n-6 and 22:6n-3 were particularly well oxidized.

Clouet *et al* (1989) have observed that the oxidation rate of 18:3n-3 was higher than that of 18:2n-6 and 18:1n-9 in isolated rat liver mitochondria. The reason was the specific activity of the enzyme carnitine palmitoyltransferase I (CPTI) measured in the mitochondrial outer membranes. Figure 7 shows that the CPTI activity towards 18:3n-3 was higher than towards 18:2n-6 and 18:1n-9 and that the activity increased with the ratio of fatty acid/albumin and more rapidly for 18:3n-3. Gavino and Gavino (1991) have confirmed these results by measuring the CPT initial velocity and additionally showed that the initial velocity was very low for 20:4n-



**Fig 7.** Carnitine palmitoyltransferase I activity measured with octadecenoic acids in outer mitochondrial membranes of rat liver at different fatty acid/albumin ratios. ■  $\alpha$ -Linolenic acid (n-3); ▲ linoleic acid (n-6), ● oleic acid (n-9). Reproduced from Clouet *et al* (1989).

6 and 22:6n-3 but relatively high for 20:5n-3, studied as acyl-CoA. They suggested that acyl-CoA synthesis may also be important in the control of the entry of fatty acids into the mitochondria, in addition to specificity of CPT1 to fatty acyl-CoA. In this view, Singh *et al* (1987) have postulated that acyl-CoA synthesis for very long-chain fatty acids was absent or was of low activity in mitochondria and for this reason, these fatty acids were directed to peroxisomal oxidation.

Factors that increase fatty-acid oxidation, in particular in peroxisomes, are likely to decrease the availability of essential fatty acids in the cell. Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase in mitochondria and any factor which decreases malonyl-CoA synthesis such as glucagon, increases mitochondrial fatty-acid oxidation. The reverse is true for insulin, which strongly stimulates malonyl-CoA synthesis and lipogenesis and decreases fatty-acid oxidation. The stimulation of peroxisomal oxidation seems to be mediated by

accumulation of fatty acyl-CoAs in the cell (Osmundsen *et al*, 1991). The activity is very sensitive to nutritional factors.

In livers of rats fed high-fat diets, peroxisomal  $\beta$ -oxidation is stimulated, in particular when the diet contains a high proportion of very long-chain fatty acids, as in hydrogenated fish oils, known to be poorly  $\beta$ -oxidized in mitochondria (Osmundsen *et al*, 1991). Polyunsaturated n-3 fatty acids are particularly potent stimulators of oxidation. Repeated administration of EPA (20:5n-3) ethyl ester to rats enhances the activity of enzymes involved in mitochondrial and peroxisomal fatty-acid oxidation (Aarsland *et al*, 1990). Willumsen *et al* (1993) have recently confirmed that prolonged administration of EPA to rats increased peroxisomal and mitochondrial fatty-acid oxidation in isolated hepatocytes. Fish oil was also observed to stimulate peroxisomal capacity to oxidize fatty acids, but not safflower oil containing nearly 85% 18:2n-6 (Halminski *et al*, 1991). Vamecq *et al* (1993) confirmed these results and considerably extended studies on regulation of fatty acid mitochondrial and peroxisomal oxidation by n-3 PUFAs. When rats were given diets enriched in n-3 and n-6 fatty acids, varying in n-3/n-6 fatty acid ratio, peroxisomal  $\beta$ -oxidation of lauroyl-CoA in liver increased with increasing ratio, being nearly 3-fold higher when the ratio increased from 0.12 to 10. In these circumstances, mitochondrial  $\beta$ -oxidation of butyryl-CoA was not increased in liver, whereas the activity of mitochondrial acyltransferases was enhanced with the higher n-3/n-6 fatty-acid ratio. In heart, the peroxisomal and mitochondrial fatty-acid oxidation was also increased but to a lesser extent and only at high ratios. Ultrastructural studies additionally showed that in hepatocytes of rats fed the high fat diet with the highest n-3/n-6 fatty-acid ratio, the number and the fractional volumes of peroxisomes and mitochondria were higher than with the lowest ratio.



These data definitely establish that dietary n-3 PUFA induce the enhancement of peroxisomal fatty acid oxidation and also mitochondrial oxidation, but less strongly. The beneficial effect of n-3 PUFA is to lower hypertriglyceridaemia, by decreasing liver VLDL secretion, in animals and humans. A possible adverse effect is to decrease the availability of n-6 polyunsaturated fatty acids (20:4n-6) reinforcing in this way the inhibiting effect of polyunsaturated n-3 fatty acids on  $\Delta 6$ - and  $\Delta 5$ -desaturation capacity of n-6 fatty acids in liver microsomes (fig 2).

In conclusion, n-6 and n-3 essential fatty acids should be provided with the diet in balanced proportion. An n-6/n-3 fatty acid ratio comprised between 4 and 6 is generally recommended as the most beneficial. They must also be provided in sufficient amounts to optimise their biological functions but not in too high amounts to avoid possible adverse effects. In healthy subjects, a dietary intake of both parent EFA (18:2n-6, 18:3n-3) of 4–6 or 7% of total energy intake could be recommended. But it depends on the other dietary components: very long-chain n-6 and n-3 fatty acids, the other dietary fatty acids, saturated, monounsaturated, *trans*-fatty acids, protein and starch *etc.* It also depends on the physiological state: pregnancy, nursing, growth or aging. Pathological states are likely to greatly modify the EFA requirements because of defects in the very-long-chain PUFA biosynthesis. However, in healthy individuals, despite the numerous factors likely to influence the availability of EFA, a regular and balanced diet usually meets the tissue requirements in n-6 and n-3 EFA.

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