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Dose-effect of dietary oleic acid: oleic acid is conditionally essential for some organs

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Abstract – The minimum dietary intake of oleic acid that is indispensable to maintain a normal content of this fatty acid in several tissues (heart, muscle, kidney and testis) was determined in the rat. For this purpose, a dose-effect study was conducted using an experimental protocol with 7 groups of rats who received a diet in which the oleic acid level varied from 0 to 6000 mg per 100 g diet, but the other ingredients were identical (in particular the essential fatty acids, linoleic and α-linolenic acid). Female rats were fed the diets from two weeks before mating, and their pups were killed aged either 21 or 60 days. When the level of oleic acid in the diet was increased, the main modifications observed in 21-day-old deficient pups were as follows: (i) for 18:1n-9, in the liver, muscle, heart, kidney, and testis, a plateau was reached at about 4 g oleic acid per 100 g diet. Below this level, the higher the dose the greater the response; (ii) for 16:1n-7, the concentration decreased in the liver, muscle, heart, kidney and testis; (iii) the concentration of 18:1n-7 decreased in the kidney, muscle, and testis; (iv) some minor modifications were noted for the other fatty acids. In mother's milk at 14 days of lactation, when dietary oleic acid increased, the levels of 18:1(n-9) also increased; the increase was regular and did not reach a plateau. In 60-day-old rats, the results were generally similar to those in 21-day-old rats, but with some differences, in particular a slight decrease in oleic acid concentration in the liver and kidney at the highest dietary oleic acid level.

oleic acid / liver / kidney / heart / muscle / testis / diet

1. INTRODUCTION

Differences in the health effects of a diet enriched in monounsaturated and polyunsaturated, as opposed to saturated fat, are due to specific fatty acid metabolism and trafficking among tissues [1]. Oleic acid (18:1n-9) is currently the subject of an important debate concerning its antiatherogenic effect [2], and it plays a role in the control of serum lipoprotein concentrations. Interestingly, oleic acid serum phospholipid content is inversely correlated with the serum total and Low Density Lipoprotein (LDL) cholesterol in elderly subjects [3, 4]. The effects of dietary oleic acid have mainly been studied in the setting of cardiovascular disease, regulation of plasma LDL and their cholesterol content and oxidizability [5–8]. Specifically in type 2 diabetic patients, an oleic acid-rich Mediterranean diet may reduce the risk of atherosclerosis by decreasing the

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level of atherogenic postprandial lipoproteins [9] and could improve glycemic control through an increase of the antidiabetic hormone glucagon-like peptide-1 [10]. Oleic acid is also involved in the modulation of blood pressure and viscosity and in the transport of cations in erythrocytes [11].

Oleic acid modulates lipid metabolism [12], it is integrated in the membrane structure and thus participates directly in the fatty acid profile of membrane phospholipids. It modulates differently the structural properties of phosphatidylethanolamine membranes in comparison to its congeners, trans-monoenic elaidate (18:1 trans Δ9) and stearate (18:0) [13]. Oleic acid affects numerous enzymatic activities of transport and receptors, as well as desaturation of essential fatty acids [14].

Even though oleic acid (and its derivatives) are present at high levels in membrane structures of all tissues, few studies have been devoted to the possible interrelations between the (n-9) (omega-9) fatty acid family and the (n-6) (omega-6) and (n-3) (omega-3) families. Nonetheless, it is known that olive oil rich in oleic acid can increase the conversion of (n-3) fatty acids [15]. However, oleic acid undergoes less oxidation in peroxisomes than polyunsaturated fatty acids [16].

The amounts of essential fatty acids, namely α-linolenic (18:3n-3) and linoleic (18:2n-6) acids, that need to be supplied in the diet to permit the elaboration and incorporation of their long-chain derivatives (arachidonic acid 20:4n-6 and docosahexaenoic acid 22:6n-3) in membrane structures in different organs have been determined [17, 18]. The amounts needed to maintain the structures in place, that is to say ensure their renewal, has also been determined [19]. The logical consequence of these results, and in view of the composition of the main commercial vegetable oils, is that it has become indispensable to determine whether dietary oleic acid affects the metabolism and incorporation of oleic acid in the membranes in the same way that it affects polyunsaturated fatty acids.

Many studies have shown the influence of the presence of a high level of oleic acid (supplied by triglycerides) on the fatty acid composition of different types of tissues and cells in numerous species [20–27], and especially of platelets [28, 29]. However, the dose-effect of dietary oleic acid on fatty acid composition has not been measured. In a previous study [30], we showed that increasing oleic acid (18:1n-9) to about a 3 g·100 g^{-1} diet enables some organs to reach nearly a normal level of 18:1(n-9) (liver, muscle, sciatic nerve), but not others (kidney, testis). It is thus evident that the organism (in particular the liver) does not have sufficient oleic acid synthesizing capability to ensure a normal composition of the membranes of some organs [30], presumably by desaturation of stearic acid (18:0) as demonstrated in cultured hepatocytes [31]. Thus, the aim of this study was to determine the minimum dietary intake of oleic acid that is indispensable in the rat to maintain a normal content of this fatty acid in several tissues (heart, muscle, kidney and testis). To determine the dose-effect of dietary oleic acid during the gestation-lactation period and in the young adult, female rats were fed the diets from two weeks before mating, and their pups were killed at the age of either 21 (weaning) or 60 days (end of growth). It was determined at which dietary concentration a plateau was reached; below this level, the higher the dose the greater the response. Since dietary deficiency of oleic acid results in certain organs not having a normal level, oleic acid is thus "conditionally essential", notably during the gestation-lactation period and in the young adult, at least in the rat.

2. MATERIALS AND METHODS

In order to determine the dose-effect of oleic acid, seven groups of female rats received semi-synthetic diets whose dietary lipids were in the form of oleisol so to

Table I. Fatty acid composition of oleisol and synthesized triglycerides trilinolein and trilinolenin used for the various diets (g·100 g^{-1} fatty acids).

	Oleisol	Trilinolein	Trilinolenin
16:0	3.1	0.3	0.0
18:0	3.0	0.4	0.2
20:0	0.3	0.0	0.0
22:0	0.6	0.0	0.0
Total saturated	7.0	0.8	0.2
16:1n-7	0.1	0.0	0.0
18:1n-9	87.2	0.7	0.6
18:1n-7	2.2	0.3	0.0
20:1n-9	0.3	0.0	0.0
Total mono-unsaturated	89.8	1.0	0.6
18:2n-6	3.0	97.5	17.7
18:3n-3	0.2	0.7	81.5

increase the oleic acid (18:1n-9) content and of pure synthetic triglycerides containing either linoleic acid (97.5% of total fatty acids) or α-linolenic acid (81.5%) in order to maintain essential fatty acid contents (Tab. I). These pure triglycerides were synthesized from these fatty acids using chemical and enzymological methods as previously described [30]. Thus with mixtures of these isocaloric dietary lipids, the diets contained increasing amounts of oleic acid (from 0 to 6000 mg·100 g^{-1} diet) and constant amounts of linoleic and α-linolenic polyunsaturated fatty acids to cover the essential fatty acid requirements (about 1.3 and 0.22 g·100 g^{-1} diet, respectively in the proportion of 1 to 6) (Tab. II).

Three weeks before mating, seven groups of Wistar female rats each received one of the seven diets. After parturition, the dams were kept in individual plastic cages. One part of their male pups were sacrificed at weaning at the age of 21 days by decapitation ($n = 5$ in each dietary group taken from at least 3 different litters). The other part of the male pups continued to receive the mother's diet and were sacrificed at 60 days of age ($n = 5$ in each dietary group). Moreover, the stomach contents of some pups killed at 14 days were collected for the analysis of fatty acid composition of the dam's milk ($n = 5$ in each dietary group). Up to 14 days after birth the diet of the pups is essentially milk, thus the analysis of their

Table II. Oil and main fatty acid contents in the seven diets differing in oleic acid content[1].

	Diet (g 18:1n-9·100 g^{-1} diet)						
	1	2	3	4	5	6	7
	Oil content g·kg^{-1} diet						
Oleisol	–	12.2	24.5	37.0	49.3	61.6	73.9
Trilinolein	13.1	13.0	12.8	12.4	12.0	11.7	11.5
Trilinolenin	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Total lipids	15.7	27.8	39.9	52.0	63.9	75.9	88.0
	mg·100 g^{-1} diet						
18:1n-9	10	1021	2040	3074	4093	5112	6131
18:2n-6	1257	1282	1299	1297	1296	1303	1320
18:3n-3	210	212	214	217	219	221	223

[1] The experimental diets contained (g·kg^{-1}) as previously described [30]: casein, 220; DL-methionine, 1.6; cellulose, 20; mineral mixture, 40; vitamin mixture, 10. According to the increasing amounts of oleisol, the cornstarch and sucrose contents were decreased from 693 g·kg^{-1} diet (diet 1: 462 g cornstarch and 231 g sucrose) to 600 g·kg^{-1} diet (diet 7: 400 g cornstarch and 200 g sucrose) [30]. Then, the caloric density was maintained between 3800 and 4066 kcal·kg^{-1} diet (15.9–17.0 MJ·kg^{-1}).

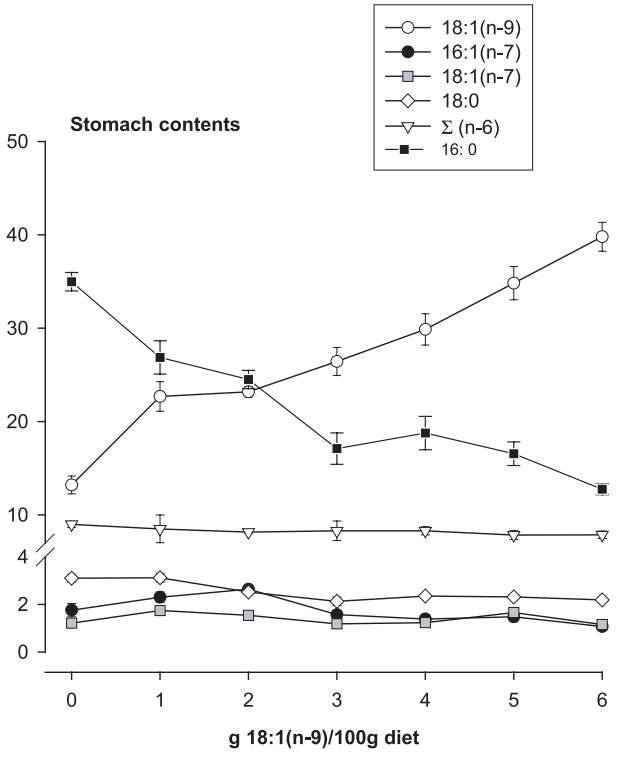

Figure 1. Effect of increasing doses of dietary oleic acid on the level of various fatty acids in the rat milk (stomach content) at day 14 of lactation.

stomach contents gives a rather precise indication of the composition of the milk they have ingested; after 14 days, the semi-synthetic diet dilutes the absorbed milk. Experimental protocols were approved and complied with government directives (Ministry of Agriculture authorization No. 03007, of 4 June 1991).

Fatty acid profiles were obtained for the stomach contents, liver, kidney, testis, muscle, and heart as previously described [17]. Briefly, lipids were extracted by the chloroform-methanol method, methyl esters were obtained by transmethylation using methanolic BF3, and fatty acid methyl esters were analyzed using gas liquid chromatography on a capillary column.

Statistical analysis was performed using the Student *t* test and ANOVA.

3. RESULTS

3.1. Stomach contents

Stomach contents reflect the composition of milk produced by the mothers and ingested by the pups. The results shown in Figure 1 are of interest. The level of 18:1n-9 in the stomach contents increased with increasing dietary oleic acid. The increase was regular and did not reach a plateau, at least not under the conditions of our experiment. In parallel to this increase, 16:1n-7 decreased and reached a plateau at 3 g oleic acid per 100 g diet. On the contrary, the level of 18:1n-7 in the stomach contents remained stable, whatever the level of dietary oleic acid. Interestingly, the 18:0 content did not increase with increasing oleic

Table III. Variations in the concentrations of fatty acids in the tissues of 21 day-old rats at dietary oleic acid levels from 0 to 6 g per 100 g diet[1].

Fatty acids	18:1n-9			16:1n-7			18:1n-7			16:0			18:0			∑ (n-6)		
g·100g^{-1}	0	6	%	0	6	%	0	6	%	0	6	%	0	6	%	0	6	%
Heart	5.6	16.5	+195	0.9	0.5	–44	5.1	4.8	–	18.3	13.9	–24	18.5	20.1	+9	40.5	32.9	–19
Liver	18.6	36.8	+98	7.6	3.3	–57	4.4	3.0	–32	29.7	22.9	–23	11.0	9.4	–15	18.9	15.8	–16
Muscle	17.6	40.9	+132	3.5	1.6	–54	3.4	2.3	–32	37.6	21.9	–42	9.7	7.2	–26	11.3	12.3	+9
Kidney	8.8	21.5	+144	1.4	0.9	–36	3.3	2.8	–15	24.3	21.9	–10	13.5	12.6	–7	35.4	28.3	–20
Testis	16.1	30.6	+90	2.2	1.4	–36	2.9	2.5	–14	32.1	25.7	–20	7.4	6.2	–16	36.5	22.8	–38

[1] Values are means of 5 determinations and are expressed in g·100g^{-1} fatty acids. % corresponds to the percentage of variation between the two extreme dietary groups. Statistical analysis showed that differences between the two dietary groups were significant for each fatty acids (Student *t* test, $P < 0.01$).

acid in the diet. The levels of 16:0 decreased considerably. Lastly, the sum of n-6 (mainly constituted of 18:2n-6) and n-3 (data not shown) did not change as a function of the level of dietary oleic acid.

For the heart, liver, muscle, kidney and testis, Table III summarizes, in 21-day-old rats, the main variations in fatty acid levels by comparing the results of animals receiving the diet deficient in oleic acid (0 g·100 g^{-1}) with those receiving the diet containing the highest level of oleic acid (6 g·100 g^{-1}). The 90 (testis) to 195 (heart) percentage increase in 18:1n-9 was compensated for by a decrease mainly of 16:0 and to a lesser extent of 18:0, 18:1n-7 and 16:1n-7. No important significantly statistical differences were found for linoleic, arachidonic, α-linolenic and docosahexaenoic acids. As shown in Table III, only the sum of (n-6) fatty acids was significantly different when oleic acid content was increased in the diet.

Figure 2 showed that in the liver, muscle, heart, kidney, and testis, a plateau was reached at about 4 g of oleic acid per 100 g of diet. Below this level, the higher the dose the greater the response.

Figure 3 and Table III shows that the concentration of 16:1n-7 decreased by 57% in the liver and 54% in muscle when dietary oleic acid increased from 0 to 6 g·100 g^{-1} of diet. At higher levels, there was a plateau. The same profile was seen for the heart, kidney, and testis, but was less marked (decrease in 44, 36, and 36%, respectively).

Figure 4 and Table III show that the concentration of 18:1n-7 decreased in the liver, muscle, kidney, and testis (32, 32, 15, and 14%, respectively) when dietary oleic acid increased from 0 to 6 g·100 g^{-1}. It stabilized at around 3–4 g·100g^{-1} diet. The heart was not affected.

Figure 5 and Table III show that an increase in dietary oleic acid induced a decrease in 16:0, in particular in the muscle (42%) and, to a lesser extent, in the heart, liver, kidney, and testis (24, 23, 10, and 20%, respectively). The increase in dietary oleic acid did not significantly affect stearic acid concentration in the organs, except in the muscle where it decreased by 26% (results not shown).

Figure 6 and Table III show that the sum of n-6 fatty acids was not constant, whatever the dose of oleic acid in the diet. The overall level decreased by 19, 16, 20, and 37% respectively in the heart, liver, kidney, and testis. In general (results not shown), the concentration of arachidonic is decreased and the concentration of 22:5(n-6) was constant in all organs, whatever the dietary level of oleic acid. However, the concentrations were lower than 2% in the heart, liver, muscle and kidney. Only the testis had a

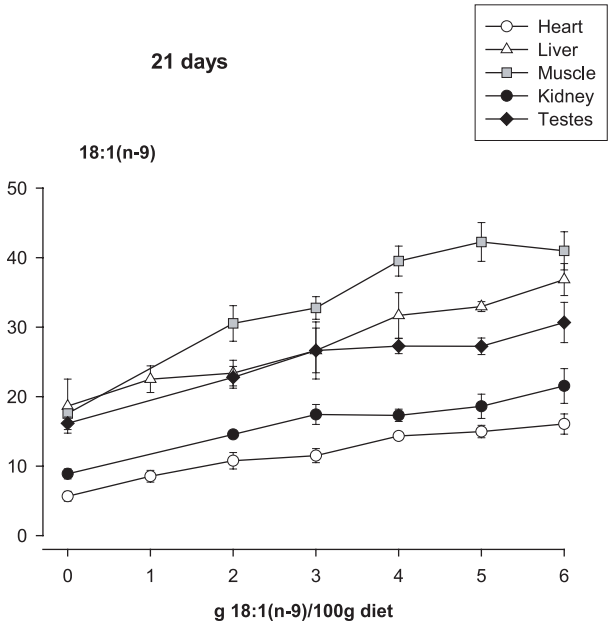

Figure 2. Effect of increasing doses of oleic acid on the level of this acid in various tissues at 21 days in the rat. The values are expressed as means ± SD (n = 5 per dietary groups).

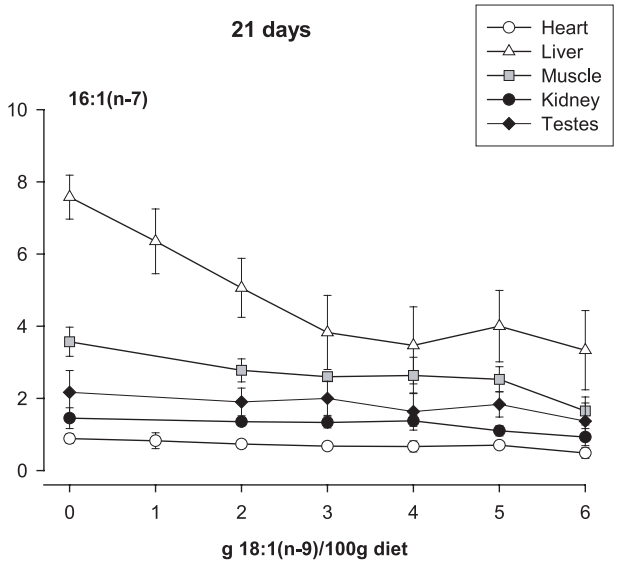

Figure 3. Effect of increasing doses of oleic acid on the level of 16:1n-7 in various tissues at 21 days in the rat. The values are expressed as means ± SD (n = 5 per dietary groups).

high level of this fatty acid. An increase in the level of dietary oleic acid did not alter the sum of (n-3) fatty acids in the groups (results not shown). In general, the concentration of 22:6(n-3) in the organs wais not modified by the level of dietary oleic acid.

3.2. Effect of increasing doses of oleic acid in 60-day-old rats

In general, notably for 16:0, 18:1n-9, 16:1n-7, and 18:1n-7, the results were similar to those in 21-day-old animals, but with

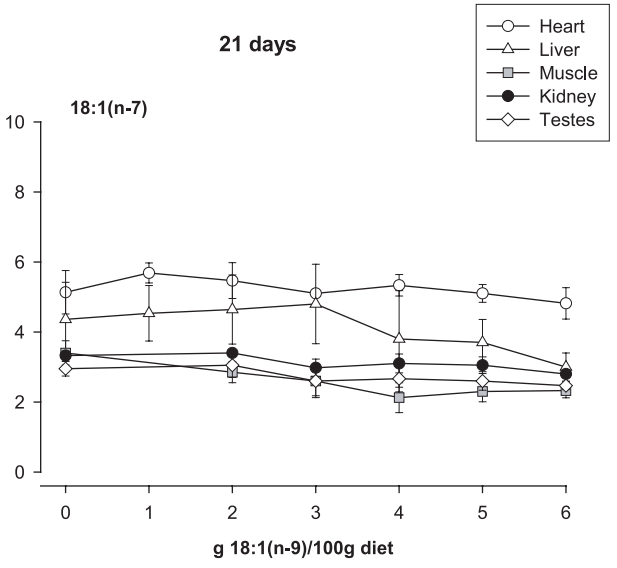

Figure 4. Effect of increasing doses of oleic acid on the level of 18:1n-7 in various tissues at 21 days in the rat. The values are expressed as means ± SD (n = 5 per dietary groups).

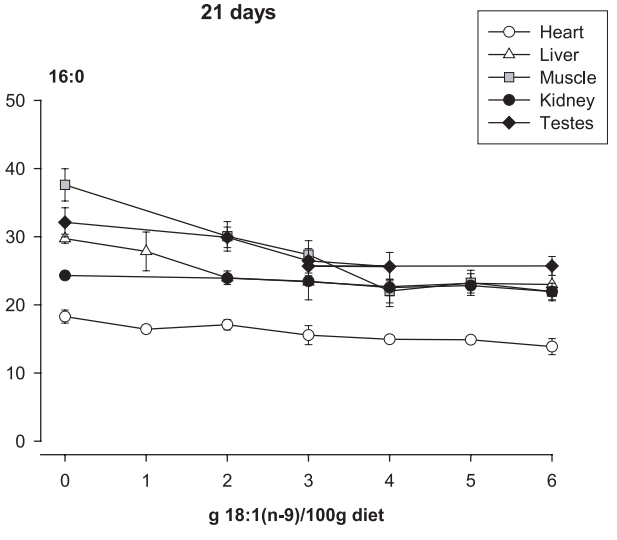

Figure 5. Effect of increasing doses of oleic acid on the level of 16:0 in various tissues at 21 days in the rat.

some differences, in particular a slight decrease in the level of oleic acid in the liver and kidney at the highest dose of oleic acid in the diet (results not shown).

4. DISCUSSION

The regular increase in oleic acid in the stomach contents, together with its plateauing in organs at 3–4 g·100 g^{-1} dietary oleic acid, suggests that excess oleic acid is used to satisfy energy needs.

However, it is indeed oleic acid itself that must be supplied by the diet. In fact, dietary supplementation with stearic acid (at two concentrations, 1.6% and 8.6%) does not modify the level of 18:1n-9 in the tissues, while the n-7 fatty acid levels remain high

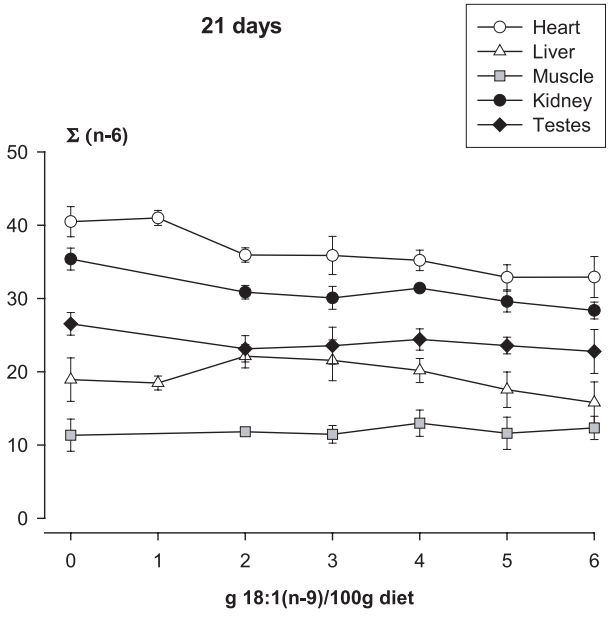

Figure 6. Effect of increasing doses of oleic acid on the level of total n-6 fatty acids in various tissues at 21 days in the rat.

[30]. Thus, delta-9-desaturase may be more active on 16:0 than on 18:0 in the synthesis of 18:1n-9 and 16:1n-7, respectively. In general, 16:1n-7 is considered to be a good indicator of lipogenesis, while palmitate is the main substance synthesized when lipogenesis is induced by carbohydrates, especially in man [32].

It should be noted that oleic acid deficiency results in a few or no changes in levels of polyunsaturated fatty acids. Moreover, dietary oleic acid has been reported to protect fatty acids of the n-3 series [15].

This dose-effect study, in which increasing amounts of oleic acid were given to different groups of animals, showed that the minimum amount needed to stabilize this fatty acid in the organs was 4 g of oleic acid per 100 g of the diet during the gestation-lactation period in 21-day-old rats. In general, the same was true for 60-day-old animals. However, high doses of dietary oleic acid seem to induce a decrease in this fatty acid in some organs such as the heart and liver.

Dietary deficiency of oleic acid results in certain organs not having a normal level of 18:1n-9. The level of 18:1n-9 in the membranes is therefore the result of both delta-9-desaturase activity and the amount of oleic acid in the diet. Oleic acid is thus conditionally essential, notably during the gestation-lactation period, at least in the rat. The relationships between the cellular and subcellular physiologies and the level of oleic acid in the membranes remain to be determined.

Thus, including oleic acid, the conditional nature of the dietary need for polyunsaturates must be re-examined: a proposal to reclassify "essential fatty acids" as "conditionally-indispensable" or "conditionally-dispensable" fatty acids [33] is interesting for oleic acid. Many organs are concerned, except the brain [34].

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