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Dietary α-linolenic acid deficiency in adult rats for 7 months does not alter brain docosahexaenoic acid content, in contrast to liver, heart and testes

Jean-Marie E. Bourre [a], Odile S. Dumont [a], Michèle J. Piciotti [a], Gérard A. Pascal [b] and Georges A. Durand [b]

[a] *INSERM U 26 Hôpital Fernand Widal, Paris (France) and* [b] *INRA CNRZ, Jouy-en-Josas (France)*

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In adult rats, 22:6($n-3$) dietary deficiency does not affect brain membranes, but has a significant effect on some other visceral organs. 60-day-old male rats fed a diet containing sufficient amounts of both linoleic and α-linolenic acid were divided into three groups. One group continued the same diet; the second was fed a diet containing 2% sunflower oil, the third was fed 10% sunflower oil (sunflower oil contains linoleic acid, but trace amount of α-linolenic acid). Animals were killed different times after receiving the new diets (1 to 31 weeks). For animals fed the diets containing only sunflower oil, deficiency in cervonic acid content (DHA, docosahexaenoic acid, 22:6($n-3$)) was not detected in whole brain, myelin or nerve endings within 31 weeks. In contrast, this acid progressively declined in liver, heart and testes up to 3 weeks and remained nearly stable thereafter. In parallel to the reduction of cervonic acid content, 22:5($n-6$) content increased in liver and heart, but not in testes. It also increased in brain, nerve endings and myelin from week 3, 6 and, 9 respectively. These results suggest that brain cervonic acid is highly preserved or is maintained at the expense of other organs.

Introduction

It is now definitively accepted that α-linolenic acid is an essential fatty acid for humans. Its deficiency alters membrane function, both in brain as well as in peripheral nerves. Previous results have shown that α-linolenic acid deficiency changes the concentrations of polyunsaturated fatty acids in membranes, with cervonic acid being replaced by 22:5($n-6$) [1,2]. Recovery from this abnormality is very slow in nervous tissue [3–6] decreased amounts of cervonic acid in brain membranes reduces enzyme activities [7], moreover 5′-nucleotidase activity is controlled by $n-3$ polyunsaturated fatty acids [8]. The electroretinogram is dramatically affected by α-linolenic deficiency in many species [7,9–12], and learning performances decline [7,13–15]. Moreover, alteration of the cervonic acid content in nerve endings changes membrane fluidity, and decreases their susceptibility to alcohol [16]. Finally, animals fed a diet deficient in α-linolenic acid become more susceptible to neurotoxins [7].

Previous studies [7] have shown that maintenance of normal cervonic acid concentrations in the lipids of all developing brain structures requires at least 0.4% calories of α-linolenic acid in the diet (lower quantities result in reduced amounts of cervonic acid in all structures examined). Thus, the demand for polyunsaturated fatty acids is very high during brain development [17–19], but it could be speculated that it decreases subsequently, as the turnover of brain structures is generally very slow.

Thus, this work was designed to determine the course of onset of the deficiency (measured by the decrease in cervonic acid and the increase in 22:5-($n-6$) content) by feeding adult animals a diet deficient in α-linolenic acid.

Material and Methods

Wistar rats were fed a semi-synthetic diet for two generations so as to stabilize fatty acid composition in all tissues. This diet contained 6% lipids (52.5% rape-

Correspondence: J.M. Bourre, INSERM U26 Hôpital Fernand Widal, 200 rue du Fb St Denis 75475 Paris cedex 10, France.

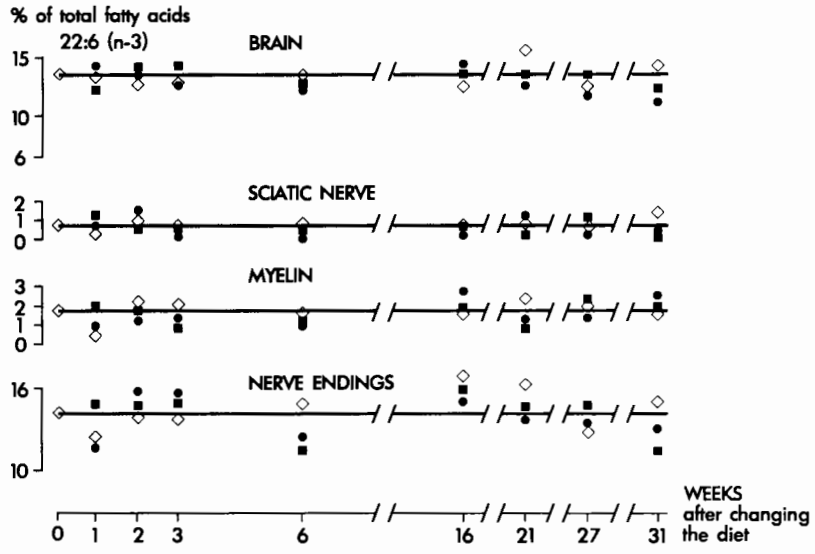

Fig. 1. Cervonic acid is maintained in nervous structures in adult rats fed a diet deficient in α-linolenic acid. y axis in the figure: % total fatty acids. ◇, control rats fed a mixture containing peanut oil and rapeseed-oil; ■, rats fed 10% sunflower oil; ●, rats fed 2% sunflower oil. Each point represents the mean value of at least three different preparations from at least four rats. Thus, each point represents at least 12 rats (from at least three different litters). For sciatic nerves and brain each point represents the mean value of at least three different preparations from at least four rats; thus, each point represents at least 12 rats (six different litters). S.D. did not esceed 10% of the mean values.

seed oil and 47.5% peanut oil), so as to provide the indispensable minimum of linoleic acid (2.4% of the calories) and α-linolenic acid (0.4% of the calories), as determined in our previous research [7]. The composition of the semi-synthetic diet has been previously published [1,7].

60-day-old rats from the third generation were divided into three groups. The first group was continued on the same diet. The second and third groups were fed diets containing, respectively, 2% providing approximately the same amount of linoleic acid as the semisynthetic diet and 10% sunflower oil. The linoleic and α-linolenic acid content of the diets are given in Table I. Animals were killed at various times after receiving the diets (1, 2, 3, 6, 16, 21, 27, 31 weeks). Various tissues were dissected out and lyophilised. Myelin and nerve endings were prepared as previously described [1]. Lipids were extracted according to the

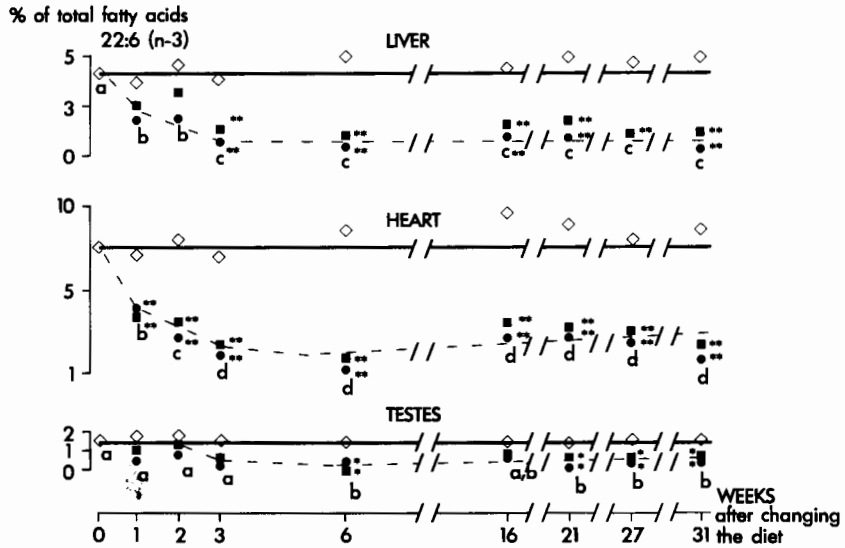

Fig. 2. Time-course of alteration of cervonic acid in various organs. Same symbols as in Fig. 1. For liver, heart, kidney, muscle, adipose tissue, each point represents the mean value of at least five different preparations from at least three rats. Thus each point represents at least 15 rats (from at least five different litters). Significant difference from controls; * $P < 0.05$; ** $P < 0.01$. Significant changes with time: values not bearing the same superscript on the same curve are significantly different at $P < 0.05$. If no superscript appears, values are not different. S.D. did not exceed 10% of the mean values. Solid line, control rats; broken line, rats fed either 2% or 10% sunflower oil.

TABLE I

Linoleic and α-linolenic content of the various experimental diets

	RP	S(2%)	S(10%)
18:2($n-6$) mg/100 g	1200	1300	6500
18:3($n-3$) mg/100 g	220	6	30
% calories	0.44	0.012	0.06

RP: mixture 52.5% rapeseed oil and 47.5% peanut oil.
S: sunflower oil.

method of Folch [20], as modified by Pollet [21]. Methylesters were obtained according to the method of Morrison [22] and analysed as previously described [1,7]. Statistical analysis was performed using analysis or variance ANOVA and Student's t-test. The number of animals is specified in the legend to the figures.

Results and Discussion

Fatty acid composition of brain, nerve endings, myelin, liver, heart and testes of 60-day-old rats fed a regular diet has been previously published [7].

For animals previously fed an α-linolenic containing diet and now fed an α-linolenic-deficient diet when 60-days-old, Fig. 1 shows that cervonic acid concentration is maintained in whole brain, myelin, nerve-endings and sciatic nerve with all diets, even the 2% sunflower oil diet. This suggests that brain cervonic acid content is highly preserved (possibly reutilized after hydrolysis of phospholipids during physiological turnover), or is maintained at the expense of the other organs.

In contrast, Fig. 2 shows that sunflower oil at both dietary levels did not provide enough α-linolenic acid to maintain the cervonic acid concentration in liver, heart and testes found in animals fed a diet containing α-linolenic acid. The cervonic acid level diminished regularly until the third week and then stabilized at 30% of control value. Interestingly, both the 6 mg and 30 mg/100 g diet levels (0.012 and 0.06% of calories) of α-linolenic acid gave identical results, suggesting that both the absolute level of α-linolenic acid and the 18:2($n-6$)/18:3($n-3$) ratio control cervonic acid in the tissues: the two diets containing sunflower oil have the same ratio, but contain different quantities of polyunsaturated fatty acids. At the same ratio, higher amounts of linoleic acid impede α-linolenic acid utilization.

Fig. 3 shows that with both sunflower oils, the 22:5($n-6$) levels were stable for 3 weeks, then it increased. For animals receiving the diet containing 10% sunflower oil, it could be due to a high level of linoleic acid in the diet, and not to α-linolenic acid deficiency. This agrees with the hypothesis that 22:5-(n–6) accumulates either when α-linolenic acid is deficient in the diet [1,2], or when linoleic acid is present in excessive amounts [23].

Accumulation of 22:5($n-6$) in brain structures was lower in this study compared to results we have obtained with animals continuously fed for several generation with the same amount of sunflower oil, showing that the plateau was not reached within the 31 weeks of this study.

Fig. 4 shows that in liver and heart 22:5($n-6$) accumulates in proportion to cervonic acid reduction as compared with Fig. 2. It was unexpected to find that 22:5($n-6$) accumulates to the same extent with sunflower oil diets containing either 1300 or 6500 mg 18:2($n-6$)/100 g diet. In these two organs, the dietary ($n-6$)/($n-3$) ratio could be a limiting factor.

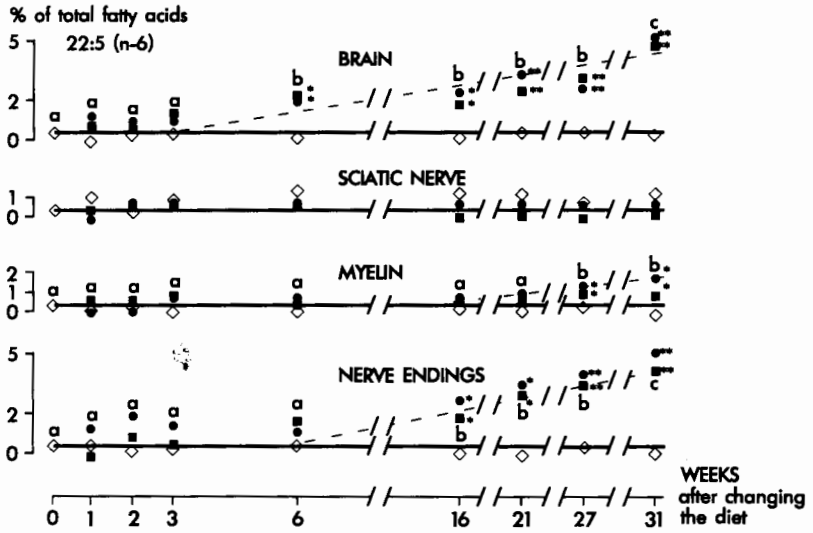

Fig. 3. Time-course of alteration of 22:5($n-6$) in nervous structures. Same remarks as in Fig. 1 and Fig 2.

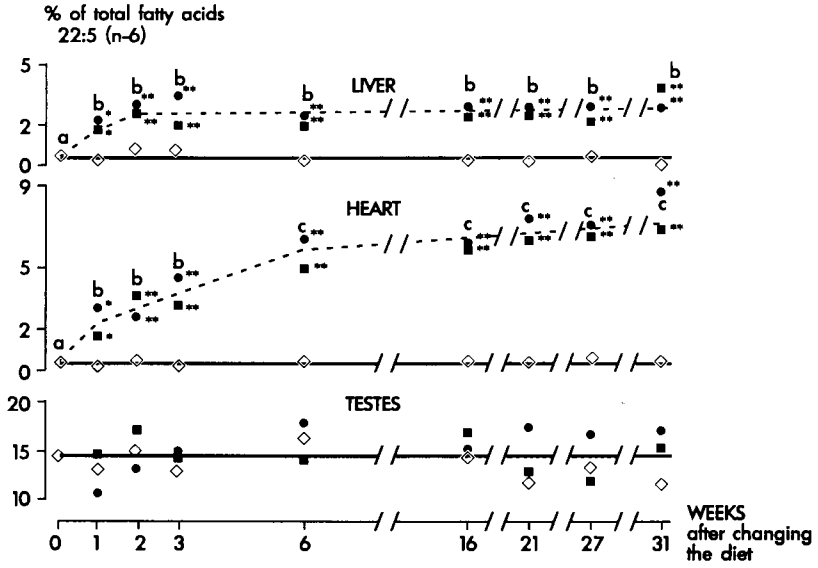

Fig. 4. Time-course of alteration of 22:5($n-6$) in various organs. Same remarks as in Fig. 2.

The 22:5($n-6$) concentration in these organs was higher than in animals fed a diet deficient in α-linolenic acid for many generations [1,7], in contrast to results obtained in brain.

In conclusion, this study shows that the content of cervonic acid is maintained for at least 31 weeks in nervous structures of normal rats, fed a diet deficient in α-linolenic acid when 60 days old. It could be proposed that utilization of α-linolenic acid is controlled not only by the dietary $(n-6)/(n-3)$ polyunsaturated fatty acid ratio, but also by the absolute levels (as suggested by this study).

We have previously demonstrated that when young animals were fed with a similar deficient diet, a marked decrease in 22:6($n-3$) and an increase in 22:5($n-6$) was noticed in the brain. In the present study with adult animals, we report that this dietary deficiency does not affect brain membranes but has a significant effect on other visceral organs. These contrasting observations obtained in the brain with age of the animals are interesting.

Acknowledgements

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