Liver Desaturase Activities and FA Composition in Monkeys. Effect of a Low-Protein Diet

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ABSTRACT: The aim of the present study was to measure Δ9-, Δ6-, and Δ5-desaturase activities in liver microsomes, as well as phospholipid FA composition of liver and erythrocytes in monkeys fed a control or low-protein diet during the postweaning period. Ten *Saimiri sciureus boliviensis* (Cebidae) of both sexes were employed; at 12 mon of age they were separated into two groups fed *ad libitum* on a control or a low-protein diet for 24 mon. *Saimiri sciureus* had active Δ9, Δ6, and Δ5 liver desaturase enzymes, and these activities were influenced by the diet. A low-protein diet produced a significant reduction in Δ5-desaturation capacity, an increase in Δ9-desaturase activity, and no change in Δ6-desaturase activity ($P < 0.05$). These changes, evoked by protein deprivation, were reflected in the liver phospholipid FA composition. Increases in the proportion of saturated FA and in monounsaturated oleic acid (18:1n-9) and a decrease in the proportion of PUFA of the n-6 and n-3 series were produced in the animals fed a low-protein diet ($P < 0.0001$). Differences between the two dietary groups were less pronounced in the FA composition of erythrocyte phospholipids.

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FA in general, and PUFA in particular, are an integral part of the structural and dynamic nature of cellular membranes on which normal physiology is crucially dependent. Moreover, PUFA play important functions as precursors of eicosanoids and as second messengers in the process of signal transduction (1,2). In animals, PUFA are synthesized from the EFA linoleic and α-linolenic acids, which are derived mainly from vegetables (1). The endogenous synthesis of PUFA starts with desaturation at the 6-position, catalyzed by the Δ6-desaturase enzyme, followed by an alternating sequence of malonyl CoA-dependent elongation and position-specific desaturation steps, in which Δ5-desaturase plays an important role (3–5). From the non-EFA palmitic and stearic acids, monounsaturated FA can be produced through the activity of the Δ9-desaturase enzyme, which introduces a *cis*-double bond at the Δ9-position.

Δ9-, Δ6-, and Δ5-desaturase activities are highly dependent on nutritional and hormonal factors (6). Previous studies carried out in our laboratory demonstrated that liver FA desaturase activities in pregnant rats were markedly affected by protein deficiency (7). The adverse effect of protein deprivation upon Δ6-desaturation activity was also demonstrated in lactating rats (8). Protein-energy malnutrition during gestation and lactation is detrimental not only to EFA metabolism but also to growth and brain development (9). In infants, the relationship between EFA metabolism and protein-energy malnutrition and the importance of dietary PUFA on visual function have been demonstrated (10,11).

Previous reports on the striking differences in desaturase activities among different animal species (12) and the lack of evidence about direct measures of liver desaturase activities in the monkey led us to study liver desaturase enzyme activities in normal monkeys as well as how they were affected after feeding on a low-protein diet.

EXPERIMENTAL PROCEDURES

Chemicals. [1-^{14}C]Palmitic (59 mCi/mmol, 99% radiochemically pure), [1-^{14}C]linoleic (55.6 mCi/mmol, 99% radiochemically pure), and [1-^{14}C]eicosatrienoic (47.0 mCi/mmol, 99% radiochemically pure) acids were purchased from New England Nuclear Corp. (Boston, MA). The pure unlabeled FFA and cofactors used for enzymatic reactions were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals and solvents were analytical grade. Solvents for HPLC were provided by Carlo Erba (Milan, Italy).

Animals and diets. Ten animals of both sexes of *Saimiri sciureus* (Cebidae) were used. They were born in captivity at the Centro Argentino de Primates (CAPRIM, Argentina). After weaning (7 mon), the animals were raised at the Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA) and were fed for 5 mon on a standard diet for their adaptation to the new environment. When the animals were 1 yr old, they were divided according to diet into two experimental groups that were fed *ad libitum* from weaning to 36 mon of age. One of them received a control diet containing 20% protein (six animals) and the other a low-protein diet containing 9% protein (four animals) (Table 1). The control and low-protein diets had the same fat content (21.6% of total calories). These diets were sufficient in EFA: They supplied linoleic acid (18:2n-6) at 11.5 mg/g and α-linolenic acid (18:3n-3) at 1.5 mg/g in the control diet, whereas in the protein-deficient diet these levels were 11.1 and 1.1 mg/g, respectively. These values represented approximately 4% and 0.4–0.5% of total calories for linoleic and α-linolenic acids, respectively. The percent distribution of FA in both diets also can be seen in Table 1. The animals were

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TABLE 1
Diet Composition

Component	Control diet[a] Amount (g)	Control diet[a] Protein content (%)	Low-protein diet[b] Amount (g)	Low-protein diet[b] Protein content (%)
Soybean meal	28.0	13.0	9.9	4.6
Wheat meal[c]	14.7	1.8	8.0	1.0
Glucose	0.0	0.0	6.7	0.0
Skim milk	10.6	3.7	4.9	1.7
Wheat bran	5.6	1.0	5.6	1.0
Sucrose	3.5	0.0	3.5	0.0
Rice meal	3.3	0.3	6.6	0.6
Cornstarch	3.0	0.0	21.4	0.0
Margarine	4.2	0.0	6.7	0.0
Egg	7.0	0.2	3.2	0.1
Vitamin mixture	1.5	0.0	1.5	0.0
Salt mixture	1.5	0.0	1.5	0.0
Water	17.1	0.0	20.5	0.0
Total	100.0	20.0	100.0	9.0

[a]FA composition of total diet: 16:0, 14.8%; 18:0, 6.7%; 18:1n-9, 26.8%; 18:1n-7, 3.8%; 18:2n-6, 41.5%; 18:3n-3, 5.6%.
[b]FA composition of total diet: 16:0, 13.9%; 18:0, 8.4%; 18:1n-9, 29.9%; 18:1n-7, 2.8%; 18:2n-6, 39.5%; 18:3n-3, 5.1%.
[c]Whole wheat flour.

weighed and sacrificed by decapitation under ether anesthesia; livers were removed, weighed, and maintained in an ice-bath until analysis.

Maintenance and treatment of the animals were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (13).

Assay for in vitro *desaturation. (i) Microsome isolation.* Livers were homogenized in 3 mL of homogenizing solution (14) for each gram of liver, and the homogenate was centrifuged at 10,000 × *g* for 20 min. The pellet was discarded, and the supernatant fraction was centrifuged again at 110,000 × *g* for 60 min in an ultracentrifuge to obtain the microsomal fraction. The entire isolation procedure was carried out at 0–4°C (14). The microsomal protein was estimated by the method of Lowry *et al.* (15).

(ii) Desaturase assays. Δ5-, Δ6-, and Δ9-desaturase activity was determined in liver microsomes by measuring the conversion of [1-^{14}C]16:0 (palmitic acid), [1-^{14}C]18:2n-6 (linoleic acid), or [1-^{14}C]20:3n-6 (eicosatrienoic acid) to [1-^{14}C]16:1 (palmitoleic acid), [1-^{14}C]18:3n-6 (γ-linolenic acid), and [1-^{14}C]20:4n-6 (arachidonic acid), respectively, according to Garda *et al.* (16). The assays were performed using 0.10 μCi of labeled acid with the corresponding unlabeled pure FA up to an amount of 16.6 nmol per tube. The FA were separated using RP-HPLC in an apparatus equipped with an L-6200 solvent delivery system, and the column eluate was monitored at 205 nm using an L-4200 UV/VIS Detector (Merck-Hitachi) as described previously (16). A 250 × 4.6 mm Econosil C18 column (Alltech Associates, Inc., Deerfield, IL) coupled to a 10 × 4 mm guard column and packed similarly was used. The flow rate was 1 mL/min. HPLC peaks were identified on the basis of their retention times relative to appropriate FA standards. Radioactivity was detected with a Radiomatic Model Flo-One/Beta radioactivity flow detector (Packard Instruments, Downers Grove, IL) using the liquid scintillation cocktail Ultima Flo-M (Packard Instruments). Desaturase activities are expressed as picomoles of [^{14}C] product of desaturation formed per minute per milligram of microsomal protein (pmol·min^{-1}·mg prot^{-1}).

Liver and erythrocyte FA analysis. Erythrocytes were separated from plasma by centrifugation. Lipids from erythrocytes and an aliquot of liver homogenate were extracted by the method of Folch *et al.* (17). The phospholipid fraction was isolated from the lipid extract by silicic acid column chromatography (Bio-Rad Laboratories, Richmond, CA) according to the method of Hanahan *et al.* (18). FAME were prepared with boron trifluoride/methanol (19) and analyzed in a chromatograph (model GC-9A; Shimadzu Corp., Kyoto, Japan) equipped with an Omegawax 250 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, PA). Authentic standards of FA were purchased from Sigma Chemical Co.

Statistical analysis. Statistical analyses were performed by one-way ANOVA. When differences were detected ($P < 0.05$), means were tested with Tukey's test (GB-STAT Professional Statistics and Graphics 4.0; Dynamic Microsystems Inc., Silver Spring, MD).

RESULTS

Figure 1 shows the growth curves for the control and low-protein-fed monkeys. From the beginning of the experiment and up to 6 mon of dietary treatment, the mean body weights were almost equivalent. However, the growth of the monkeys fed

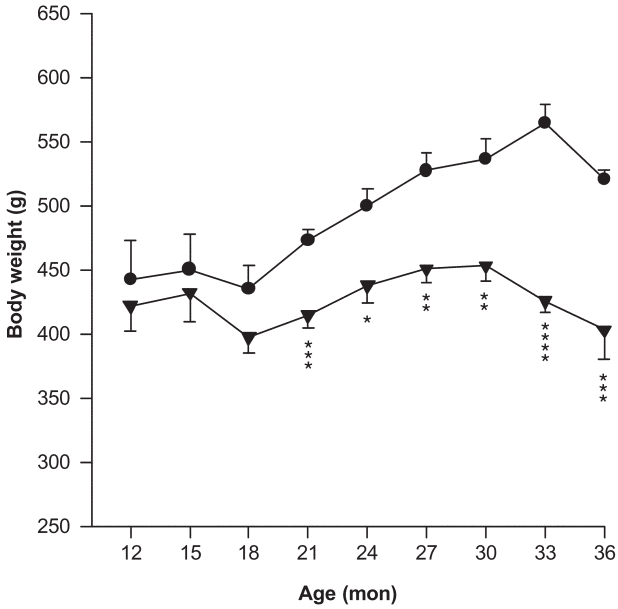

FIG. 1. Body weights of monkeys fed control (●; $n = 6$ animals) and low-protein (▼; $n = 4$) diets recorded during the entire experimental period. Values are means ± SEM. Significant differences relative to control group are indicated by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

on a low-protein diet were retarded, and the body weights were significantly lower than the control ones. Differences between the two groups increased with age. Liver weights from the control group also were significantly higher than those of the low-protein-fed group (12.2 ± 0.6 vs. 9.7 ± 0.7 g, $P < 0.05$).

From the results shown in Table 2, it is clear that *S. sciureus* have active Δ9-, Δ6-, and Δ5-desaturase enzymes. This fact was demonstrated by the conversion of the respective radioactive precursors into their desaturated products by the three enzymes in the liver microsomal fraction during the postweaning period. Moreover, these desaturase activities were influenced by nutritional factors. The highest value was obtained for the Δ5-desaturase, followed by those for Δ9- and Δ6-. The low-protein diet had different effects on the desaturase activities; a significant reduction in Δ5-desaturation capacity was observed in the animals fed a 9% protein diet compared with the control ones. On the other hand, an increase in Δ9-desaturase activity was obtained when animals were fed the low-protein diet. However, in our experimental conditions, Δ6-desaturase activity was not modified according to the diet protein level.

The changes observed in the desaturation activities evoked by dietary protein level in monkeys were reflected in the liver phospholipid FA composition (Table 3). A significant increase in the proportion of saturated FA of 12 and 14 carbons as well as in the monounsaturated oleic acid (18:1n-9) was found in low-protein-diet animals compared to the control. A marked decrease in the proportion of PUFA of the n-6 series, such as 20:2n-6, 20:4n-6, and 22:4n-6, and in the major component of the n-3 series, 22:6n-3, in monkeys fed on a low-protein diet relative to the control was also shown. These results evidenced a significant decrease in the total PUFA, especially those of the n-3 series in the low-protein-fed group when compared to the control one. On the contrary, the sum of total n-9 FA was higher in the phospholipid fraction of animals fed a low-protein diet than that from controls. Table 3 also shows the effect of the deficient diet on the unsaturation index calculated in the total phospholipid fraction, which was significantly diminished compared to the controls.

In the present study we also analyzed the FA composition of erythrocyte phospholipids from monkeys (Table 4). Differences between the two dietary groups were smaller than those found in the liver phospholipid fraction. A significant increase in the proportion of monounsaturated 18:1n-9 acid as well as in the 20:5n-3 level was detected in the erythrocyte phospholipid fraction compared with the control. On the contrary, a significantly lower proportion of 22:6n-3 was observed in monkeys fed a low-protein diet compared with the controls.

TABLE 2
Desaturase Activities of Liver Microsomes from Monkeys Fed on Control and Low-Protein Diet (pmol·mg prot^{-1}min^{-1})[a]

Desaturase enzymes	Substrate/product	Control diet ($n = 6$)	Low-protein diet ($n = 4$)
Δ5	20:3n-6/20:4n-6	88.0 ± 4.0	71.7 ± 3.5*
Δ6	18:2n-6/18:3n-6	29.6 ± 1.8	27.2 ± 2.5
Δ9	16:0/16:1	40.6 ± 3.6	72.0 ± 3.5**

[a]Values are mean ± SEM of *n* determinations. Probability value relative to control monkey data. *$P < 0.05$; **$P < 0.0001$.

TABLE 3
FA Composition[a] (mol%) of Liver Phospholipid from Monkeys Fed on Control and Low-Protein Diets

FA	Control diet ($n = 6$)	Low-protein diet ($n = 4$)
12:0	0.28 ± 0.03	1.78 ± 0.05***
14:0	0.25 ± 0.07	1.22 ± 0.16***
16:0	19.12 ± 0.87	19.16 ± 0.33
16:1n-7	0.60 ± 0.08	0.78 ± 0.24
16:2n-4	0.65 ± 0.14	0.43 ± 0.01
18:0	21.94 ± 0.81	21.24 ± 0.92
18:1n-9	4.72 ± 0.36	7.25 ± 0.50**
18:1n-7	2.48 ± 0.25	2.90 ± 0.81
18:2n-6	20.85 ± 1.15	21.68 ± 0.56
18:3n-6	0.22 ± 0.02	0.17 ± 0.02
18:3n-3	0.28 ± 0.02	0.23 ± 0.03
20:0	0.15 ± 0.01	0.13 ± 0.01
20:1n-9	0.15 ± 0.02	0.22 ± 0.04
20:2n-6	0.82 ± 0.02	0.52 ± 0.02***
20:3n-6	1.41 ± 0.09	1.56 ± 0.14
20:4n-6	14.57 ± 0.37	13.42 ± 0.24*
20:3n-3	0.19 ± 0.04	0.18 ± 0.02
20:5n-3	0.19 ± 0.02	0.06 ± 0.03
22:1n-9	0.16 ± 0.01	0.70 ± 0.03***
22:4n-6	1.74 ± 0.10	0.74 ± 0.06***
22:4n-3	1.51 ± 0.10	0.84 ± 0.12**
22:5n-6	1.00 ± 0.08	1.06 ± 0.13
22:5n-3	0.49 ± 0.08	0.37 ± 0.10
22:6n-3	6.20 ± 0.32	4.33 ± 0.22**
Σ Saturated	42.01 ± 1.65	43.87 ± 0.92
Σ Monounsaturated	8.10 ± 0.58	11.85 ± 1.14
Σ Polyunsaturated	50.10 ± 0.56	45.59 ± 0.53***
Σ n-9	5.03 ± 0.38	8.17 ± 0.49***
Σ n-6	40.60 ± 1.15	39.15 ± 0.42
Σ n-3	8.85 ± 0.42	6.01 ± 0.32***
Unsaturation Index	177.13 ± 4.43	157.34 ± 1.27**

[a]Values are mean ± SEM of *n* determinations. Some minor components have been omitted. Probability values relative to data from control monkeys: *$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$.

DISCUSSION

The number of FA double bonds is an important factor in determining membrane structure, packing, fluidity, and function. Δ9-Desaturase is widely distributed in different kinds of animals. However, there is considerable variation between animal species in their ability to synthesize the C_{20} and C_{22} PUFA. High rates of desaturation are observed in small species, particularly the rat (20,21).

It was reported that some animals, notably cats and lions, which are carnivores, have a very limited ability to synthesize C_{20} and C_{22} PUFA and consequently have a strict requirement for a dietary source of preformed C_{20} and C_{22} PUFA (22–25). However, recent reports showed that cats would be able to convert 18:2n-6 and 18:3n-3 to longer-chain PUFA after feeding on an EFA-deficient diet for 6 mon (26).

Although indirect evidence has been reported in favor of the presence of Δ6- and Δ5-desaturases in monkeys, an indirect

TABLE 4
FA Composition (mol%) of Erythrocyte Phospholipid from Monkeys Fed on Control and Low-Protein Diets[a]

FA	Control diet (n = 6)	Low-protein diet (n = 4)
12:0	0.09 ± 0.03	0.13 ± 0.11
14:0	0.19 ± 0.03	0.28 ± 0.02
16:0	22.13 ± 0.34	21.82 ± 0.93
16:1n-7	0.23 ± 0.06	0.51 ± 0.03
16:2n-4	0.52 ± 0.01	0.42 ± 0.02
18:0	18.00 ± 0.29	17.75 ± 0.37
18:1n-9	10.26 ± 0.31	11.90 ± 0.11*
18:1n-7	5.59 ± 0.53	4.75 ± 0.61
18:2n-6	21.56 ± 0.41	21.16 ± 0.73
18:3n-6	0.08 ± 0.04	0.17 ± 0.05
18:3n-3	0.05 ± 0.02	0.06 ± 0.02
20:0	0.44 ± 0.02	0.52 ± 0.01
20:1n-9	0.48 ± 0.03	0.35 ± 0.05
20:2n-6	0.84 ± 0.04	0.56 ± 0.04**
20:3n-6	0.70 ± 0.07	0.87 ± 0.06
20:4n-6	9.34 ± 0.31	9.50 ± 0.22
20:5n-3	0.32 ± 0.05	0.59 ± 0.03*
22:1n-9	0.41 ± 0.08	0.20 ± 0.06
22:4n-6	2.16 ± 0.18	2.78 ± 0.17
22:4n-3	1.33 ± 0.08	1.30 ± 0.03
22:5n-6	0.75 ± 0.05	1.11 ± 0.11
22:5n-3	0.21 ± 0.03	0.35 ± 0.02
22:6n-3	3.88 ± 0.11	2.31 ± 0.08**
Σ Saturated	41.28 ± 0.45	41.25 ± 1.34
Σ Monounsaturated	16.97 ± 0.83	17.71 ± 0.73
Σ Polyunsaturated	41.67 ± 0.75	41.11 ± 0.75
Σ n-9	11.15 ± 0.33	12.45 ± 0.20
Σ n-6	35.36 ± 0.63	36.15 ± 0.81
Σ n-3	5.80 ± 0.19	4.54 ± 0.08**
Unsaturation Index	147.67 ± 2.22	145.48 ± 2.44

[a]Values are mean ± SEM of n determinations. Some minor components have been omitted. Probability values relative to data from control monkeys: *$P < 0.01$; **$P < 0.001$.

measurement of these enzymatic activities has been published. Su *et al.* (27) have reported that fetal organs from pregnant baboons who consumed a long-chain PUFA-free diet and received [U-^{13}C]linoleic acid in their third trimester of gestation could accumulate 18:2 acid within a day of a maternal dose and convert much of it to 20:4 within weeks (27). Another approach to the study of desaturase activities in monkeys was made by Kanazawa *et al.* (28), who studied the synthesis of chain elongation–desaturation products from linolenate by brain microsomes of Japanese monkeys *Macaca fuscata fuscata,* demonstrating that it was markedly influenced by age. However, in none of these works was the contribution of each desaturase in the final conversion of linoleic or α-linolenic acid to arachidonate or docosahexaenoate, respectively, clearly determined, and the rate of conversion was very low.

On the other hand, previous research carried out in our laboratory demonstrated that liver FA desaturating activities were profoundly affected by protein deficiency in pregnant rats; this fact would alter the normal supply of PUFA to the fetus (9). An alteration in the FA composition was also observed in malnourished nursing infants compared to normal ones (10,29). However, in these cases the direct measurement of desaturase activities could not be carried out. In postweaning monkeys fed on a low-protein diet, we observed changes in liver and erythrocyte FA composition similar to the ones in malnourished infants (10,29), as well as an increment in saturated and monounsaturated acid with a concomitant decrease in PUFA from the n-6 and n-3 series (Tables 3 and 4). Although we could not observe any alteration in Δ6-desaturase activity, we were able to demonstrate that the changes in FA composition in monkeys fed a low-protein diet correlated with a significant decrease in Δ5- and an increase in Δ9-desaturase. All these results would imply an important contribution to the study of the desaturase activities in primates since they could be directly measured in an animal model closely related to the human.

This is the first direct measurement of desaturase activity in primates, and these enzymatic activities were found to depend on the diet. Moreover this kind of monkey responded to the low-protein diet in the same way as already observed in other mammals in spite of the evolutionary difference between rats and humans.

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