Relationship between rat liver microsomal Δ6 and Δ5 desaturase activities and fatty acid composition: comparative effects of coconut and salmon oils during protein restriction

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The aim of this work was to compare the effects of coconut and salmon oils on rat liver microsomal Δ6 and Δ5 desaturations, during protein restriction. A higher Δ6 desaturase activity was noted in rats fed the low-protein coconut oil diet, in comparison with that occurring in rats fed either a low-protein or normal-protein salmon oil diet. No variation was observed in Δ5 desaturase activity or in 20:4n-6/18:2n-6 ratio. The fatty acid composition of liver microsomal phospholipids provided evidence of higher levels of 20:5n-3 and 22:6n-3 in the normal-protein salmon oil group, when compared with the low-protein salmon oil group. No influence of experimental diets on the total n-3 and total n-6 fatty acids could be demonstrated. Aside from investigating the effects of protein restriction on the liver microsomal desaturases, this work shows that there is no correlation between microsomal desaturation rates and microsomal phospholipid profiles even when diets are rich in polyunsaturated fatty acids (salmon oil).

Keywords: protein restriction; salmon oil; coconut oil; liver desaturases; fatty acid composition

Introduction

Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are the main dietary essential fatty acids (EFA). In the liver, linoleic acid is converted mainly into arachidonic acid (20:4n-6) and α-linolenic acid into eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3).[1]

Numerous studies on desaturases and protein malnutrition have been performed: Narce et al.[1] studied the time-course effect of protein restriction on hepatic linoleic acid Δ6 and Δ5 desaturations in the growing rat. These activities were decreased to 20%–30% of their original values after 2 days of low-protein (LP) diet, but after 25 days of LP diet, the Δ6 desaturase activity increased to 91% of the original values, whereas the Δ5 desaturase activity represented only 70%. These authors (unpublished data), have reported that the Δ9 desaturase activity was decreased after 2 and 14 days of LP diet, by 33% and 55%, respectively, in the same experimental conditions.

Fish oil, soybean protein, and age interfere with Δ6 desaturation of linoleic acid.[2,3] The combined effects of these factors on fatty acid metabolism were assessed by Choi et al.[4] on rats fed purified diets containing 20% protein either as casein or soybean protein with 5% sardine oil. A significant effect of the soybean protein diet on fatty acid profiles of liver microsomal phospholipids with an increase in 18:2n-6 was observed when compared with the casein diet. This dietary protein effect was still present when fish oil was used as a source of fat.

It is well known that 18:3n-3 competes with 18:2n-6 for Δ6 desaturation.[5] Eicosapentaenoic acid

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(20:5n-3) also competes with linoleic acid for Δ6 desaturation.[6]

Williams and Hurlebaus[7] showed in the rat that a dietary protein deficiency may lead to changes in lipid composition and suggested it might be attributable to an impairment of the enzyme systems converting linoleic into arachidonic acid. It has been reported that proteins are important for lipoprotein synthesis thus for transport and metabolism of essential fatty acids (EFA). Moreover, Hill and Holman[8] showed that during protein restriction when protein levels are low protein deficiency increases the EFA requirements, involving a decreased availability of EFA.

Therefore, the combined influence of dietary protein levels (as 20% or 2% casein) associated with dietary saturated (coconut oil) or polyunsaturated (salmon oil) fatty acids was examined directly on linoleic Δ6 and Δ5 desaturations. As protein restriction impairs fatty acid composition of rat liver membrane phospholipids,[7,9] the relationships between desaturase activities and microsomal fatty acid composition were investigated.

Material and methods

Animals and diets

Sixteen male Wistar rats (Iffa-Credo, l'Arbresle, France), weighing 80 ± 5 g at the beginning of the experiment were fed a balanced diet (20% casein + 5% olive oil) for 10 days until they reached an average body weight of 112 ± 13 g. They were then randomized into four equal groups and fed the diets reported in *Table 1*. Two groups continued to receive the same diet in which 5% coconut oil (NPc) or salmon oil (NPs) replaced olive oil. The two other groups were fed a low-protein diet containing 2% casein and 5% coconut oil (LPc) or salmon oil (LPs). The rats were kept in wire-bottom cages at constant temperature (25° C) and humidity (60 ± 5%) with a 07:00 a.m. to 07:00 p.m. light cycle. Diets and tap water were supplied ad libitum for 28 days. The fatty acid composition of the two experimental oils are shown in *Table 2*.

Table 1 Composition of diets[a]

Diet	Normal Protein (20% casein) (g/kg)	(kJ/kg)	Low Protein (2% casein) (g/kg)	(kJ/kg)
casein[b]	200	3420	20	342
D,L methionine	3	50	3	50
corn starch[c]	587	10060	767	13140
sucrose	50	850	50	850
cellulose[b]	50	—	50	—
mineral mixture[d]	40	—	40	—
vitamin mixture[e]	20	—	20	—
coconut,[f] salmon oil[g]	50	1900	50	1900

[a] Both diets are semi-synthetic, isoenergetic (16280 kJ/kg diet), and were given as powder.
[b] Prolabo Paris, France
[c] Etablissements Louis François S.A., 94100 Saint Maur, France
[d] UAR 205 B (Villemoisson), 91360 Epinay/Orge, France. The salt mixture provides the following amounts in mg/kg of diet: $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4$, 7 H_2O, 200; trace elements, 400; completed to 40,000 with cellulose. Trace element mixture (mg/kg of diet): $MnSO_4$, H_2O, 98; $CuSO_4$, 5 H_2O, 20; $ZnSO_4$, H_2O, 80; $CoSO_4$, 7 H_2O, 0.1; KI, 0.3.
[e] UAR 200 vitamin mixture provides the following amounts per kg of diet: retinol, 39,600 IU; cholecalciferol, 5000 IU; thiamin, 40 mg; riboflavin, 30 mg; pantothenic acid, 140 mg; pyridoxine, 20 mg; inositol, 300 mg; cyanocobalamin, 0.1 mg; ascorbic acid, 1600 mg; α tocopherol, 340 mg; menadione, 80 mg; nicotinic acid, 200 mg; choline, 2720 mg; folic acid, 10 mg; p aminobenzoic acid, 100 mg; biotin, 0.6 mg; completed to 20,000 with cellulose.
[f] Astra-Calvé, Asnières, France
[g] Gattefossé, 69804 Saint Priest Cedex, France

Chemicals

[1-^{14}C]α-Linolenic, [1-^{14}C]linoleic and [2-^{14}C]dihomo-γ-linolenic acid, (56 mCi/mmol, 97.5% radiochemical purity) were purchased from the Radiochemical Centre (Amersham, UK). Each substrate was diluted in ethanol with the corresponding unlabeled pure fatty acid to a specific activity of 10 mCi/mmol. Unlabeled fatty acids, factors and biochemicals were provided by Sigma Chemical Co. Inc (St. Louis, MO, USA).

Isolation of microsomes

The rats were killed by exsanguination between 07:00 a.m. and 08:00 a.m. Liver tissue was quickly removed, cut into thin slices, homogenized at 4° C in a Potter-Elvejhem homogenizer with 6 volumes of 0.05 M phosphate buffer (pH 7.4) and 0.25 M sucrose solution, and centrifuged at 13,000*g* for 20 min. The microsomes were then isolated from the supernatant fraction by centrifugation at 105,000*g* for 60 min. The microsomal pellets were resuspended in the 0.05 M phosphate buffer (pH 7.4) and 0.25 M sucrose solution, and the protein content was determined by the method of Layne.[10]

Incubations of microsomes

Five mg microsomal proteins were incubated in an open flask with saturating quantities of substrate:[11] 120, 120, and 80 nmoles for [1-^{14}C]18:3n-3, [1-^{14}C]18:2n-6 and [2-^{14}C]20:3n-6, respectively. Incubations were performed at 37° C in a shaking water bath for 15 min in a total volume of 2.1 mL incubation medium, containing in mmol/L: phosphate buffer (pH 7.4) 72, $MgCl_2$ 4.8, coenzyme A 0.5, ATP 3.6, and NADPH 1.2. Incubations were stopped by adding 15 mL chloroform/methanol (1:1, vol/vol), saponified, and methylated according to Slover and Lanza.[12] The conversion of labeled substrates into their Δ6n-3, Δ6n-6, and Δ5n-6 desaturation products (stearidonic, γ-linolenic, and arachidonic acids, respectively) was determined by the reversed phase HPLC method described by Narce et al.,[13] using a Waters (Waters Associates, Milford, MA, USA) chromatograph and a Lichrocart column (Superspher RP 18, 250 mm × 4 mm i.d.; Merck-Clevenot, Marne, France).

Fatty acid analysis

Lipids from aliquots of liver microsomes were extracted according to Delsal[14] and phospholipids were obtained by the method described by Hirsch and Ahrens.[15] After adding heptadecanoic acid (used as internal standard), phospholipids were saponified and methylated according to Slover and Lanza,[12] then analyzed by capillary gas-liquid chromatography (Becker-Packard model 417 gas-liquid chromatograph equipped with a 50 m capillary glass column packed with Carbowax 20 M; Packard Instrument Co., Downers Grove, IL, USA).

Table 2 Fatty acid composition (% moles) of coconut and salmon oils

Fatty acid	Coconut oil	Fatty acid	Salmon oil
8:0	4.0	14:0	2.0
10:0	6.0	16:0	12.0
12:0	39.0	16:1n-7	6.0
14:0	19.0	18:0	4.0
16:0	16.0	18:1n-7	
16:1n-7	tr[a]	+18:1n-9	18.0
18:0	4.6	18:2n-6	4.0
18:1n-9	8.9	20:1n-11	
18:2n-6	1.6	+20:1n-9	10.0
18:3n-6	tr	20:4n-6	1.1
$>C_{20}$	tr	20:5n-3	9.9
		22:1n-11	
		+22:1n-9	8.0
		22:5n-3	3.9
		22:6n-3	11.1
		24:1n-9	1.2
Total SFA[b]	88.6	Total SFA	18.0
Total PUFA[c]	10.5	Total PUFA	36.0
PUFA/SFA	0.1	PUFA/SFA	2.0
		n-6/n-3	0.7

[a] tr, traces
[b] SFA, saturated fatty acids
[c] PUFA, polyunsaturated fatty acids

Stastical analysis

All data are presented as the means ± SE. After analysis of variance using a Duncan's multiple range test, means were compared according to the least significant difference ($P < 0.05$).

Results

Table 3 represents the general characteristics of the rats. After 28 days of LP diet, in LPc and LPs groups, body weights were 45% and 46% and liver weights were 66% and 46%, respectively, of NPc and NPs values, respectively. Moreover, a lipidic effect was observed because liver weights of the LPs group were significantly lower than those of the LPc group.

In *Table 4*, the results are expressed as percentage of substrate converted after Δ6 and Δ5 desaturations. Rats fed the low-protein coconut oil diet (LPc) showed a higher Δ6 desaturation than those of LPs and NPs groups.

Table 5 reports the fatty acid composition of liver microsomal phospholipids. A higher proportion of total n-3 fatty acids was observed in the liver of rats fed the salmon oil diet. The major n-3 fatty acids obtained, were as follows: 20:5n-3 (EPA) and 22:6n-3 (DHA). Evidence of a higher level of 20:5n-3 and a lower level of 22:4n-6 in NPs group was provided when these values were compared to the LPs group. Percentages of total n-3 fatty acids were the same in NPc and LPc groups, and in NPs and LPs groups. There was no difference in the 20:4n-6 to 18:2n-6 ratio values in any of the four groups of rats.

Table 3 Body weight, liver weight*, food intake and liver lipid content†

	20% casein		2% casein	
Experimental oil	coconut (NPc)	salmon (NPs)	coconut (LPc)	salmon (LPs)
Body weight (g)	283 ± 7[a]	270 ± 8[a]	129 ± 6[b]	124 ± 12[b]
Liver weight (g)	8.7 ± 0.5[a]	9.5 ± 0.9[a]	5.7 ± 0.3[b]	4.4 ± 0.5[c]
Food intake (g/day)	22.1 ± 1.7[a]	20.5 ± 1.3[a]	10.7 ± 0.5[b]	9.9 ± 0.8[c]
Food intake (g/100g Bw)	7.97	8.87	7.20	7.66
Lipid content (mg/g liver)	58.7 ± 4.4[a]	104 ± 11[b]	68.3 ± 1.4[c]	145 ± 13[d]

* Results are means ± SE for n = 4 animals per group.
† Results are means ± SE for n = 4 animals, during 28 days of diet. After analysis of variance (Duncan's multiple range test), means were compared in each experimental diet group according to the least significant difference and classified according to decreasing order. In each line, means assigned different superscript letters were significantly different ($P < 0.05$).

Table 4 Desaturase activities in liver microsomes*

Desaturation quantity of substrate	Δ6 18:3n-3 18:4n-3 120 nmoles	Δ6 18:2n-6 18:3n-6 120 nmoles	Δ5 20:3n-6 20:4n-6 80 nmoles
Diet	Percentage of conversion (%)		
20% casein			
coconut oil (NPc)	13.4 ± 2.3[ab]	6.7 ± 0.8[b]	13.5 ± 1.7[a]
salmon oil (NPs)	12.8 ± 1.4[b]	7.2 ± 0.8[b]	12.9 ± 1.9[a]
2% casein			
coconut oil (LPc)	15.9 ± 1.3[a]	9.0 ± 0.7[a]	13.9 ± 2.1[a]
salmon oil (LPs)	11.4 ± 0.4[b]	8.0 ± 1.9[ab]	14.4 ± 2.8[a]

* Results are means ± SE for n = 4 animals per group.
After analysis of variance (Duncan's multiple range test), means were compared in each experimental diet group according to the least significant difference and classified according to decreasing order. In each column, means assigned different superscript letters were significantly different ($P < 0.05$).

Discussion

The food intake was higher in the rats fed coconut oil (NPc and LPc), compared with the salmon oil fed rats (NPs and LPs). Expressed per day and per 100 g bodyweight, the food intake was similar for the four groups (*Table 3*). Therefore both 2% casein groups suffered from protein restriction and not from protein-energy malnutrition.[1] The growth retardation of malnourished rats is a more sensitive indicator of their protein status

Table 5 Fatty acid composition (% moles) of liver microsomal phospholipids*

Fatty acid	20% casein		2% casein	
	coconut oil (NPc)	salmon oil (NPs)	coconut oil (LPc)	salmon oil (LPs)
16:0	18.06 ± 0.38^{c}	20.26 ± 0.33^{b}	23.74 ± 0.71^{a}	23.80 ± 1.06^{a}
16:1n-7	2.28 ± 0.22^{a}	2.25 ± 0.29^{a}	2.00 ± 0.09^{a}	1.34 ± 0.24^{b}
18:0	21.65 ± 0.72^{a}	17.15 ± 1.07^{b}	19.67 ± 0.13^{a}	17.80 ± 0.77^{b}
18:1n-9	7.14 ± 0.62^{b}	6.12 ± 0.30^{b}	12.33 ± 0.40^{a}	7.97 ± 1.57^{b}
18:1n-7†	4.50 ± 0.17^{a}	4.74 ± 0.87^{a}	—	—
18:2n-6	8.44 ± 0.53^{a}	4.33 ± 0.56^{b}	6.58 ± 0.74^{a}	4.02 ± 0.69^{b}
18:3n-6	0.26 ± 0.06^{a}	0.04 ± 0.01^{c}	0.12 ± 0.04^{b}	0.07 ± 0.01^{bc}
18:3n-3	0.10 ± 0.03^{a}	0.12 ± 0.04^{a}	0.05 ± 0.01^{a}	0.07 ± 0.02^{a}
20:3n-6	1.74 ± 0.11^{a}	0.78 ± 0.09^{b}	1.75 ± 0.14^{a}	0.68 ± 0.08^{b}
20:4n-6	21.95 ± 1.30^{a}	12.26 ± 0.72^{b}	21.28 ± 0.91^{a}	10.68 ± 0.83^{b}
20:5n-3	0.35 ± 0.02^{c}	15.44 ± 1.49^{a}	0.26 ± 0.02^{d}	6.18 ± 0.95^{b}
22:4n-6	0.37 ± 0.21^{a}	0.05 ± 0.01^{b}	0.37 ± 0.05^{a}	0.10 ± 0.01^{b}
22:5n-6	1.47 ± 0.22^{a}	0.19 ± 0.03^{c}	1.40 ± 0.08^{a}	0.33 ± 0.02^{b}
22:5n-3	0.33 ± 0.03^{c}	3.08 ± 0.17^{a}	0.54 ± 0.08^{b}	3.23 ± 0.24^{a}
22:6n-3	6.13 ± 0.52^{c}	11.60 ± 0.81^{b}	6.73 ± 1.05^{c}	23.56 ± 1.81^{a}
SFA‡	40.14 ± 0.76^{c}	37.57 ± 0.79^{d}	43.94 ± 0.74^{a}	41.84 ± 0.39^{b}
MUFA§	14.29 ± 0.67^{a}	14.03 ± 1.00^{a}	14.43 ± 0.33^{a}	9.69 ± 1.71^{b}
Total n-6	38.61 ± 1.22^{a}	17.94 ± 1.27^{c}	33.48 ± 1.66^{b}	15.96 ± 0.77^{c}
Total n-3	6.91 ± 0.56^{b}	30.24 ± 1.04^{a}	7.56 ± 1.09^{b}	33.00 ± 2.43^{a}
20:4n-6 / 18:2n-6	2.61 ± 0.23^{a}	2.87 ± 0.29^{a}	3.27 ± 0.35^{a}	2.76 ± 0.60^{a}

* Results are expressed as molar percentage of fatty acid. Results are means ± SE for n = 4 animals per group. After analysis of variance (Duncan's multiple range test), means were compared in each experimental diet group according to the least significant difference and classified according to decreasing order. In each line, means assigned different superscript letters were significantly different ($P < 0.05$).
† Peak values inferior to 0.05% are not reported.
‡ SFA, saturated fatty acids
§ MUFA, mono-unsaturated fatty acids

than the measurement of tissue protein contents.[1] Except for the liver weight of the LPc group, the relative decrease in weight was the same for the liver and the whole body. This decrease is an adaptive response to protein depletion: in these conditions, protein requirements of the lean body mass diminished.[1]

The duration of 28 days for the low-protein diet was established according to the results of Narce et al.[1] who showed that after 25 days of LP diet, Δ6 and Δ5 desaturase activities were close to control values, thus revealing an adaptive response of desaturases to protein restriction.

Protein deficiency leads to a decrease in liver microsomal phospholipid C_{20} and C_{22} fatty acids, particularly 20:4n-6 accompanied by an accumulation of 18:2n-6,[1] even if the liver lipid content is increased in the low-protein groups (*Table 3*). We explain this result by the fact that during protein restriction an impaired transport of triacylglycerols by very-low density lipoprotein (VLDL) from the liver is responsible for the hepatic steatosis (increased liver lipid content),[16,17] even if the amount of fatty acids compared with others decreased (*Table 5*). De Tomas et al.[18] evidenced a low 20:4n-6 to 18:2n-6 ratio after a partial protein deprivation; this might be due to a reduction in enzyme activities involved in 20:4n-6 biosynthesis from 18:2n-6. As protein diet does not influence the elongation step,[19] Δ6 desaturase activity impairment might be responsible for these changes. Our results (*Table 4*) show a higher percentage of Δ6 desaturation with LPc diet than with NPc diet. As previously reported,[1] our results confirm that adaptive changes take place during long-term protein restriction; however in our experimental conditions Δ6 desaturase activities are increased significantly with LPc diet. After an adaptation to malnutrition, the pool of circulating amino acids decreased, as they are used more efficiently for synthesis.[1] While the animals adapted themselves to the LP diet, their growth was retarded and protein catabolism and amino acid availability increased. These effects may provide an explanation for high desaturase activities in LPc group.

If desaturase activities are not significantly affected by the origin of the oil added to the 20% casein diet, Δ6n-3 desaturase activity was lower when rats were fed the LPs diet than when they received the LPc diet. It can be hypothesized that high amounts of 20:5n-3 (salmon oil) could inhibit Δ6n-3 desaturation by negative feedback effect. The amount of 20:5n-3 provided by salmon oil is sufficient to inhibit the Δ6n-3 desaturase activity. However, no influence of this fatty acid was noted in the NPs group. This result can be explained by the fact that in rats fed low-protein diets, the liver lipogenic enzymes, and especially desaturases, are decreased by a failure in DNA, RNA, and protein synthesis.[20,21] Thus, the quantity of 20:5n-3 is

sufficient to inhibit Δ6n-3 desaturase activity. In this case desaturase activity becomes the limiting factor for the rats fed the LPs diet.

The 18:3n-3 fatty acid is a more efficient substrate for liver Δ6 desaturase activities than 18:2n-6, as reported by Sanders and Rana,[5] and it is not affected by protein malnutrition.[5,22]

Liver microsomal total n-6 and n-3 polyunsaturated fatty acid (PUFA)[5] were altered by providing salmon oil (NPs and LPs) (*Table 5*). The LP coconut diet influenced the PUFA level, specifically total n-6 fatty acids, which were decreased when SFA increased and MUFA remained stable, compared with the NPc group. After 28 days of LPc diet, the percentage of 18:1n-9 increased. This may be due in part to an increase in Δ9 desaturase activity, which is affected differently by diet when compared with other desaturases.[23] However Narce et al. (unpublished data), showed that Δ9 desaturase activity was also decreased by LP diet. The composition reported on *Table 5* reflects the n-3 fatty acid supply brought by salmon oil (EPA and DHA) that represents, with arachidonic acid, the major PUFA contained in this oil.

20:4n-6 to 18:2n-6 ratio (*Table 5*) does not account for the modifications observed in the proportion of these two fatty acids and the variations in Δ6 desaturase activity as shown in *Table 4*. The results of the present study indicate no correlation between the changes in desaturase activities and microsomal fatty acid composition, as previously described in other experimental conditions.[24] A decrease in the relative amounts of n-6 PUFA appears when an increase in n-3 fatty acids is obtained in liver microsomal phospholipids. These modifications may induce a higher fluidity of the microsomal membranes (n-3 PUFA), but they are not related to desaturase activities. Indeed, the bioconversion of 18:2n-6 into 20:4n-6 and of 18:3n-3 to its LC-PUFA could also depend on other interacting factors, including elongation, oxidation, substrate availability, removal of product, and lipid class fatty acid incorporation, as well as dietary fats. However, previous studies[9] have explained nonparallelism between desaturase activities and fatty acid composition by liver lipid composition that can change progressively when rats are fed a low-protein diet for a 6-week period. The time-course of the protein restriction effects on desaturation and microsomal lipid composition may vary, explaining why no correlation was observed between these two parameters. These results show that desaturase activities are not the essential factor for liver microsomal fatty acid composition.

It can be concluded that in our experimental conditions salmon oil, associated with low protein diet, influences only Δ6n-3 desaturation, compared with coconut oil. It can hence be stated that even if PUFA are incorporated into microsomal membranes, they have no effect on desaturases but the latter might benefit from the resulting fluidity to move out of the internal layer membrane and be therefore more accessible to fatty acids.

Abbreviations

EFA	essential fatty acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
HPLC	high-performance liquid chromatography
LC-PUFA	long-chain polyunsaturated fatty acid
LP	low-protein diet
NP	normal-protein diet
MUFA	mono-unsaturated fatty acid
SFA	saturated fatty acid
VLDL	very-low density lipoprotein

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