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Fatty acid profiles in subcutaneous and mesenteric adipose tissues from Zucker rats after energy restriction. Influence of dietary fat

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Several disturbances concerning lipid metabolism are found in obese Zucker rats. The present study was designed to determine the effect of energy restriction on fatty acid profile of adipose tissue triglycerides from two different anatomic locations, in genetically obese rats. The impact of the addition of a high amount of olive oil to a restricted diet was also considered. Lean and obese male Zucker rats, 11 weeks old, were used. The former (n = 7) were fed a control diet *ad libitum* during 4 weeks, and the latter (n = 21) were randomized into 3 groups and fed either a control diet *ad libitum* or diets restricted to 75 % of lean rat intake which provided different amounts of olive oil as fat source. Obesity induced strong modifications in fatty acid profiles from subcutaneous and mesenteric adipose tissues that were not corrected when a standard-fat 25 % energy-restricted diet was used for feeding. In contrast, when a high amount of olive oil was included into the restricted diet, a new fatty acid profile in adipose tissue from obese rats, that mirrors the one of lean rats, was observed. Significant differences between two anatomic locations were found in the pattern of modifications produced by obesity, but not by dietary treatments.

Key words: Genetic obesity, Energy restriction, Adipose tissue fatty acids, Olive oil.

The *fa/fa* Zucker rat is a model of genetic obesity and hyperlipemia, which has proven to be a reasonable animal model for early-onset human obesity, as it shares many traits with human obesity syndrome (23).

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Several disturbances concerning lipid metabolism are found in obese Zucker rats (5, 20, 37). In this context, abnormal polyunsaturated fatty acid metabolism can be identified in 12-day-old rats, a really early stage in the development of their obesity (29).

Adipose tissue is generally considered to be a storage organ for energy excess. The fatty acid composition of adipose tissue is partly dependent on dietary intake (2, 13, 22), but other factors must also be taken into account. Thus, endogenous synthesis of non-essential fatty acids via hepatic lipogenesis and metabolic conversions occurring between different fatty acids can be contributing factors to adipose tissue composition (4, 36). Finally, the selective release of certain fatty acids during lipid mobilization induced by a negative energy balance or physical activity must be considered (16, 32, 35).

The fatty acid composition of animals seems to play a role in their environmental adaptation. For example, the adipose tissue composition of some hibernating animals may influence their ability to tolerate the required low temperature state. It also varies depending on the site, to accommodate to different temperature extremes experienced there (15, 30).

The present study was designed to determine the effect of energy restriction on fatty acid profile of adipose tissue triglycerides, from two different anatomic locations, in genetically obese rats. The impact of the addition of a high amount of olive oil was also considered.

Materials and Methods

Animals, diets and experimental design.– Seven male lean (*Fa/?*) Zucker rats (11 weeks old) and twenty-one male obese (*fa/fa*) Zucker rats (11 weeks old) were purchased from Iffa-Credo (Barcelona, Spain). They were adapted to the room and cage environments 1 week before the beginning of the protocol. All animals were housed individually in polycarbonate metabolic cages (Tecniplast, Gazzada, Italy) and maintained at a temperature (23 ± 2 °C) and humidity (50 %) controlled room with 12 h-12 h light-dark cycle, lights on at 08.00 h.

During 4 weeks, lean rats (AL) were fed a control diet *ad libitum*. Obese rats were randomized into three groups: a group fed a control diet *ad libitum* (AF) and two groups fed defined diets restricted to 75 % of the AL group energy intake (RF, RFO). Food intakes of lean freely fed animals (AL) were monitorized daily; the mean group food intake was calculated and appropriate quantities of diets were offered to each restricted group in order to provide identical energy to the two treated groups. Diets were freshly prepared every week throughout the experimental period and stored at –20 °C to avoid rancidity. The composition of diets is described in table I. Amounts of casein, methionine, cellulose, minerals and vitamins were adjusted to ensure adequate intake of these nutrients when diets were consumed at the 75 % energy level. All animals had free access to water. Fatty acid content of olive oil was analyzed by gas chromatography (total saturates, monounsaturates and polyunsaturates: 13.7 %, 74.8 % and 11.0 %, respectively).

Casein was purchased from Sigma (Barcelona, Spain), starch from Vencasser (Bilbao, Spain), cellulose from Fluka (Barcelona, Spain) and vitamins from Roche (Barcelona, Spain). Olive oil was obtained from local sources.

At the end of the feeding period, rats were killed by decapitation after an overnight fast (12 h) and total subcutaneous and mesenteric adipose tissues were dissected, weighed, immediately frozen in

Table I. *Composition of experimental diets (g/kg diet).*

	Dietary groups*		
	AL and AF	RF	RFO
Casein[1]	140	190	240
Methionine	5	7	9
Saccharose	350	310	160
Wheat starch	350	310	160
Olive oil	50	40	250
Coconut oil	–	–	–
Cellulose	50	68	85
Mineral mix[2]	45	61	77
Vitamin mix[3]	10	13	17
Choline chloride	1	1	2
Energy content (kJ/kg diet)	15 959	15 079	18 821

*Dietary groups: AL, lean rats fed *ad libitum;* AF, fatty rats fed *ad libitum;* RF, fatty rats fed a standard-fat energy-restricted diet; RFO, fatty rats fed a high-olive oil energy-restricted diet.

[1]Casein 90 %; [2]Mineral mix (g/kg): NaCl 139.3, K_2HPO_4 389.1, $CaCO_3$ 381.4, $MgSO_4.7H_2O$ 57.3, $FeSO_4.7H_2O$ 27.0, $MnSO_4.H_2O$ 4.0, $ZnSO_4.7H_2O$ 1.25, KI 0.8, $CuSO_4.5H_2O$ 0.5, $CoCl_2.6H_2O$ 0.02; [3]Vitamin mix (mg/g): Retinol 120, Cholecalciferol 2.5, α-Tocopherol acetate 3,000, Phylloquinone 5, Thiamin 600, Riboflavin 600, Niacin 2,000, Pyridoxine 600, Folic acid 100, Cyanocobalamin 5.4, Calcium pantothenate 1,000. Sufficient lactose (carrier) was added to make up 1 g.

liquid nitrogen and stored at –20 °C until analysis.

Fatty acid composition of diets and adipose tissues.– Small fragments (1 g) of subcutaneous and mesenteric fat pads were rinsed of red blood cells, melted at 70 °C and filtered. The lipid extracts and the olive oil were transmethylated with methanol in sulphuric acid. Analysis of fatty acids was conducted using a Perkin Elmer 8310 gas chromatograph equipped with a flame ionization detector and a 2 m column packed with 10 % DEGS-PS on 80/100 chromosorb (Supelco; Sugelabor). Nitrogen was used as the carrier gas. The temperature of the oven and the injection port were maintained at 170 °C and 225 °C respectively. Peaks were identified using authentic fatty acid methyl ester standards obtained from Sigma (Barcelona, Spain). For estimation of total fatty acid content, the areas under the peaks were measured using an integrator (Perkin-Elmer GP-100). All samples were analysed in quintuplicate. The replicate error (CV) was 5% or less of the mean for all fatty acids.

Statistics.– Values presented in tables and figures are given as the mean of each set of seven animals. Variability was estimated as the standard error of the mean (SEM). Data were subjected to ANOVA analysis. When significant effects were found, Duncan's *t* test was used to determine the significance of differences between experimental groups, as well as to analyze differences between the two tissues in the pattern of response to obese syndrome and to dietary treatments. In all statistical analysis differences were considered significant at $p < 0.05$.

Results

Fatty rats fed *ad libitum* (AF) showed a greater body weight due to an increase of adiposity, as shown by adipose tissue

weights (table II). Energy restriction resulted in significantly decreased subcutaneous adipose tissue weight in the two groups compared with AF group. However, values remained clearly higher than those of lean rats (AL). Subcutaneous depot was more sensitive than mesenteric fat pad, losing 21 % and 14 % in RF, and RFO groups respectively *vs* AF group.

Table III summarizes the mean percentages for each fatty acid measured and total percentages of saturated, monounsaturated and polyunsaturated fatty acids in subcutaneous adipose tissue. Marked differences in fatty acid profile were found between fatty rats (AF) and their lean counterparts (AL). Main changes consisted in a 20 % increase in total saturated fatty acid content, due to the higher amounts of 14:0 and 16:0, and a decrease in polyunsaturated fatty acids; in AF group, 18:2*n*-6 was reduced to 60 % of the amount found in AL group and 18:3*n*-3 fell to undetectable values. Concerning monounstaurated fatty acids, each one was differently modified. The percentage of 14:1*n*-5 and those of 16:1*n*-7 were about 2-fold greater in AF rats compared with AL rats, whereas 18:1*n*-9 showed a decrease of about 14 %.

Energy restriction was not effective to reverse the alterations of adipose tissue fatty acid composition shown by fatty rats as no significant differences were found between AF and RF groups.

When a high amount of olive oil was incorporated into the energy-restricted diet (RFO), there were a decrease in total saturated fatty acids ($p = 0.0018$) and an increase in total monounsaturated and polyunsaturated fatty acids ($p = 0.0019$ and $p = 0.0268$, respectively) compared with values of AF rats. Proportions of 18:1*n*-9 and 18:3*n*-3 became similar to those of lean rats. The modified percentages of other fatty acids were also affected but they did not reach the values of lean rats.

Correlation values between dietary fatty acids and fatty acid profile in this depot were AL = 0.9680, AF = 0.8944, RF = 0.8939 and RFO = 0.9508.

Fatty acid profile of mesenteric adipose tissue triglycerides is shown in table IV. Fatty rats (AF) presented a higher content of saturated fatty acids (30 %) and a smaller content of monounsaturated (7 %) and polyunsaturated (27 %) than lean counterparts. In subcutaneous adipose tissue as well as in mesenteric depot no changes were found when fatty rats were fed an energy-restricted diet, except in 18:1*n*-9. However, when feeding fatty animals an olive oil supplemented energy-restricted diet, several modifications were observed. Saturated fatty acids were sig-

Table II. *Body and adipose tissue weights (g) of animals fed the experimental diets,* ad libitum *or at 25 % energy restriction.*

Data are the mean ± SEM of seven values. Letters represent comparison among experimental groups. Data not sharing the same letter are statistically different, $p < 0.05$.

Dietary groups*	AL	AF	RF	RFO
Body wt				
Initial	275 ± 4.00[a]	344 ± 6.00[b]	347 ± 6.00[b]	340 ± 7.00[b]
Final	336 ± 7.00[a]	391 ± 4.00[b]	337 ± 4.00[a]	349 ± 5.00[a]
Adipose tissue wt				
Subcutaneous	6.90 ± 0.67[a]	55.07 ± 1.63[b]	43.64 ± 1.68[c]	47.51 ± 1.91[c]
Mesenteric	2.00 ± 0.22[a]	7.54 ± 0.49[b]	7.25 ± 0.18[b]	7.53 ± 0.26[b]

*Dietary groups: AL, lean rats fed *ad libitum;* AF, fatty rats fed *ad libitum;* RF, fatty rats fed a standard-fat energy-restricted diet; RFO, fatty rats fed a high-olive oil energy-restricted diet.

Table III. *Trglyceride fatty acid composition of subcutaneous adipose tissue from animals fed the experimental diets,* ad libitum *or at 25 % energy restriction.*
Data are the mean ± SEM of seven values and are expressed as g/100 total fatty acids. Letters represent comparison among experimental groups. Data not sharing the same letter are statistically different, $p < 0.05$.

Dietary groups*	AL	AF	RF	RFO
12:0	0.4 ± 0.1^a	0.3 ± 0.1^a	0.4 ± 0.1^a	0.3 ± 0.1^a
14:0	1.7 ± 0.1^a	2.0 ± 0.1^b	2.3 ± 0.1^b	1.5 ± 0.1^c
14:1*n*-5	0.2 ± 0.1^a	0.4 ± 0.1^b	0.4 ± 0.1^b	0.3 ± 0.1^c
16:0	22.3 ± 0.6^a	27.8 ± 0.2^b	27.9 ± 0.3^b	24.7 ± 0.2^c
16:1*n*-7	6.6 ± 1.0^a	13.0 ± 0.3^b	12.5 ± 0.2^b	8.7 ± 0.3^c
18:0	2.4 ± 0.1^a	2.1 ± 0.1^b	2.0 ± 0.1^b	2.1 ± 0.1^b
18:1*n*-9	55.3 ± 0.8^a	47.8 ± 0.2^b	47.5 ± 0.4^b	55.3 ± 0.4^a
18:2*n*-6	9.5 ± 0.6^a	5.7 ± 0.2^b	5.5 ± 0.2^b	6.1 ± 0.2^b
18:3*n*—3	0.3 ± 0.1^a	$< 0.1^b$	$< 0.1^b$	0.3 ± 0.1^a
Σ Saturates	26.8 ± 0.5^a	32.1 ± 0.2^b	32.7 ± 0.3^b	28.6 ± 0.3^c
Σ Monounsaturates	62.8 ± 0.5^a	61.2 ± 0.3^{ab}	60.4 ± 0.3^b	64.3 ± 0.5^c
Σ Polyunsaturates	9.8 ± 0.5^a	5.7 ± 0.2^b	5.6 ± 0.2^b	6.4 ± 0.2^c

*Dietary groups: AL, lean rats fed *ad libitum;* AF, fatty rats fed *ad libitum;* RF, fatty rats fed a standard-fat energy-restricted diet; RFO, fatty rats fed a high-olive oil energy-restricted diet.

Table IV. *Triglyceride fatty acid composition of mesenteric adipose tissue from animals fed the experimental diets,* ad libitum *or at 25 % energy restriction.*
Data are the mean ± SEM of seven values and are expresed as g/100 total fatty acids. Letters represent comparison among experimental groups. Data not sharing the same letter are statistically different, $p < 0.05$.

Dietary groups*	AL	AF	RF	RFO
12:0	0.2 ± 0.1^a	0.2 ± 0.1^a	0.2 ± 0.1^a	0.2 ± 0.1^a
14:0	1.7 ± 0.1^a	2.0 ± 0.1^b	2.0 ± 0.1^b	1.5 ± 0.1^c
16:0	22.2 ± 0.6^a	29.4 ± 0.1^b	29.9 ± 0.2^b	25.8 ± 0.4^c
16:1*n*-7	6.3 ± 0.2^a	12.4 ± 0.2^b	12.3 ± 0.2^b	8.6 ± 0.1^c
18:0	2.3 ± 0.1^a	2.7 ± 0.1^b	2.8 ± 0.1^b	2.7 ± 0.1^b
18:1*n*-9	58.2 ± 0.7^a	47.3 ± 0.2^b	46.3 ± 0.4^c	54.3 ± 0.5^d
18:2*n*-6	6.9 ± 0.4^a	5.0 ± 0.2^b	5.0 ± 0.2^b	5.6 ± 0.2^b
Σ Saturates	26.5 ± 0.6^a	34.2 ± 0.1^b	34.9 ± 0.2^b	30.2 ± 0.4^c
Σ Monounsaturates	64.5 ± 0.6^a	59.7 ± 0.2^b	58.6 ± 0.4^b	62.9 ± 0.5^c
Σ Polyunsaturates	6.9 ± 0.4^a	5.0 ± 0.2^b	5.0 ± 0.2^b	$5.6^b \pm 0.2^b$

*Dietary groups: AL, lean rats fed *ad libitum;* AF, fatty rats fed *ad libitum;* RF, fatty rats fed a standard-fat energy-restricted diet; RFO, fatty rats fed a high-olive oil energy-restricted diet.

nificantly reduced ($p = 0.0011$) although they remained 14 % higher than those of lean rats. In contrast, monounsaturated fatty acids were significantly increased ($p = 0.0012$) because of the strong enrichment of adipose tissue with 18:1*n*-9. Polyunsaturated fatty acids were not affected.

Correlation values between dietary fatty acids and fatty acid profile in this

depot were AL = 0.9711, AF = 0.8815, RF = 0.8729 and RFO = 0.9423.

In order to study differences in the pattern of response between the two adipose anatomical locations considered in this work of obese syndrome, percentages of modification for each fatty acid were calculated using lean rats (AL) as controls. Differences in the pattern of response to dietary treatments were assessed using untreated fatty rats (AF) as reference group in order to obtain the modification percentages.

Discussion

Data presented in this work show that body weight losses can be induced by energy restriction in genetically obese rats. At the age of 15 weeks, fatty treated rats (RF) reached similar body weight to lean littermates. Weight reduction was in part explained by the decrease of fat subcutaneous accumulation. An important reduction of fat free mass was also observed (8; data not shown in this work).

The study was focused on the possible modifications induced by energy restriction in adipose tissue fatty acid profiles. To have reference groups, fatty acids in adipose tissues of untreated lean and obese rats were determined.

The analysis of adipose tissue fatty acid composition showed that genetic obesity induces strong alterations in fatty acid metabolism. Thus, an increase in saturated fatty acids and a decrease in monounsaturated and polyunsaturated fatty acids were observed. The results are in good accordance with data presented by PHINNEY *et al.* (28). A possible explanation for these alterations is a preferential incorporation of some saturated fatty acids into adipose tissue triglycerides as a result of the lipoprotein-lipase activity and/or the endogenous fatty acid synthesis (1).

On the other hand, in mesenteric adipose tissue 18:0 increased in 14.8 % and 18:1*n*-9 was reduced in 18.7 %. It could be proposed, therefore, that Δ9 desaturase enzyme activity, which allows the synthesis of 18:1*n*-9 from 18:0, could be impaired in this adipose location.

To investigate the ability of energy restriction to correct the alterations induced by genetic obesity in triglyceride fatty acid profiles from adipose tissue, fatty rats were fed a restricted diet (75 % of the AL energy intake).

In contrast with several works, where changes in the partitioning of fatty acid oxidation are shown (10-12,21,32), in our study four weeks of 25 % energy restriction, which produced a mild reduction of mesenteric adipose tissue (10 %; $p = 0.10$) and a significant reduction of subcutaneous fat pad (21 %; $p = 0.0026$), did not induce modifications in fatty acid profiles of adipose tissue, regardless of the anatomical location, indicating that there was not a selective mobilization or retention of some fatty acids when a negative energy balance was induced. These discrepancies can be attributed to the different strain of rats. Thus, whereas JONES *et al.* (17) used Sprague-Dawley rats weighing 193 g and RACLOT *et al.* (32) used Wistar rats weighing 200 g, we carried out the study in genetically obese rats, weighing approximately 340 g. On the other hand, experimental design was also different. Thus, in the study of RACLOT *et al.* (32), lipolysis was induced under *in vitro* conditions and an energy-restriction was not imposed. In the published bibliography, there are also many controversial reports when adipose tissue fatty acid composition was compared before and after weight loss in human subjects. Thus, ROSSNER *et al.* (33) showed no significant changes in any of the measured fatty acids. In contrast, HUDGINS and HIRSCH (16) found small but significant changes in 16:0

and 18:2*n*-6 fatty acids. Both studies differed in energy restriction level.

It has been proposed that fatty acid composition of tissues in human subjets and animals can be determined, in some extent, by the type of dietary fat consumed. Many studies have been published to show the presence of correlationship between dietary fatty acids and fatty acid profiles in different tissues (11, 14, 18, 22, 26, 27, 31). In this context, the research has been frequently focused on long-chain polyunsaturated fatty acids of the *n*-3 and *n*-6 series. Recently, great attention has been paid to the intake of olive oil with its high oleic acid (18:1*n*-9) and moderate linoleic acid (18:2*n*-6) levels.

A question that can arise is whether dietary modifications concerning amount and fatty acid composition of fat could modify fatty acid profile of adipose tissue, when a negative balance is induced by food deprivation and if it could, therefore, be possible to reestablish the fatty acid profile found in lean rats by dietary treatments. To investigate this aspect, obese rats were fed a diet which provided the same energy as RF diet, i.e. 25 % energy restriction *vs* AL group, now containing a high amount of olive oil as source of monounsaturated fatty acids. In this diet fat represented 40 % of consumed energy.

Under those conditions, the fatty acid composition of adipose tissue triglycerides was greatly affected by dietary lipid manipulation. Thus, fat pads from rats fed an enriched olive oil diet showed an important increase in oleic acid, according to the high proportion of this fatty acid (73.4 %) in the dietary fat. This effect compensated, totally in the subcutaneous depot and partially in the mesenteric adipose tissue, the reduced level of oleic acid found in AF rats.

Since energy intake in RFO group was identical to that of RF group, changes observed in this group were likely due to the influence of dietary fat. These changes led to new triglyceride fatty acid profiles which mirror, in some extent, fatty acid composition of diets, as shown by correlation coefficient values.

In sumary, accretion of fatty acids from adipose tissue seems to be particularly sensitive to genetic obesity. In contrast, in genetically obese rats, fatty acid release induced by energy restriction from fat depots does not modify fatty acid profiles. Finally, lipid metabolism of genetically obese rats may be modulated by the high-fat diets depending on the nature of the fatty acids, which, in turn, may determine the way the tissues respond to endocrine and regulatory stimuli. It must be emphasized that most of the modifications induced by feeding RFO diet make *fa/fa* rat fatty acid profile very similar to that of lean rats.

Results only show site-specific differences in the pattern of modification induced by obesity but not in the pattern of response to dietary treatment, because significant differences in fatty acid profiles were found between subcutaneous and mesenteric adipose tissues when AF rats were compared with AL rats (saturates $p = 0.0001$, monounsaturates $p = 0.0001$, polyunsaturates $p = 0.0002$). Differences in fatty acid profiles between subcutaneous and mesenteric adipose tissues have been well documented (7, 25).

M. P. PORTILLO, R. CANTORAL, M. I. TORRES, M. A. DE DIEGO y M. T. MACARULLA. *Perfil de ácidos grasos en tejido adiposo de ratas Zucker tras restricción energética. Influencia de la grasa de la dieta.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (3), 317-326, 1997.

En ratas Zucker obesas se observan numerosas alteraciones que afectan al metabolismo de los lípidos. El objetivo de este trabajo es determinar el efecto de la restricción energética sobre el perfil de ácidos grasos de los triglicéridos presentes en el tejido adiposo de dos loca-

lizaciones anatómicas diferentes. Se considera también la posible influencia de la adición de una elevada cantidad de aceite de oliva a la dieta hipocalórica. Se utilizan ratas macho Zucker, obesas y no obesas, de 11 semanas de edad. Ratas no obesas (n = 7) se alimentan *ad libitum* con una dieta control durante 4 semanas, y ratas obesas (n = 21), repartidas en tres grupos, se alimentan *ad libitum* con una dieta control o con una restricción del 25 % respecto a la ingesta de las ratas control no obesas. Las dietas de restricción energética aportan cantidades diferentes de aceite de oliva como fuente lipídica. La obesidad produce importantes alteraciones en el perfil de ácidos grasos de los depósitos adiposos subcutáneo y mesentérico. Estas modificaciones no son corregidas tras la alimentación con una dieta de restricción calórica cuyo aporte graso representa el 12 % de la energía total. Por el contrario, al incluir una elevada cantidad de aceite de oliva en una dieta hipocalórica, se observa un nuevo perfil de ácidos grasos en el tejido adiposo de las ratas control no obesas. Existen diferencias significativas entre las dos localizaciones anatómicas estudiadas en cuanto a las alteraciones producidas por la obesidad, pero no en la respuesta a los tratamientos dietéticos.

Palabras clave: Obesidad genética, Restricción energética, Acidos grasos de tejido adiposo, Aceite de oliva.

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References

1. Bath, B. G., Xia, T., Mostafa, N., Florant, G. L. and Coleman, R. A. (1992): *FASEB J.*, 6, A1672 (abstr).
2. Berry, E. M., Hirsch, J., Most, J., McNamera, D. J and Thorton, J. (1985): *Am. J. Clin. Nutr.*, 44, 220-231.
3. Bourre, J. M. E., Dumont, O. L., Clément, M. E. and Durand, G. A. (1997): *J. Nutr.*, 127, 488-493.
4. Bray, G. A. (1969): *Proc. Soc. Exp. Biol. Med.*, 131, 1111-1114.
5. Bray, G. A. (1977): *Fed. Proc.*, 36, 148-153.
6. Brenner, R. R. (1984): *Progr. Lip. Res.*, 213, 69-96.
7. Calder, P. C., Harvey, D. J., Pond, C. M. and Newsholme, E. A. (1992): *Lipids*, 27, 716-720.
8. Cantoral, R., De Diego, M. A., Macarulla, M. T. and Portillo, M. P. (1996): *Int. J. Obes.*, 20 (Suppl. 4), 148.
9. Cha, M. C. and Jones, P. J. (1996): *Nutr. Biochem.*, 7, 650-658.
10. Chen, Z. Y. and Cunnane, S. C. (1992): *Am. J. Physiol.*, 263, R233-R239.
11. Cunnane, S. C., McAddo, K. R. and Horrobin, D. F. (1986): *Br. J. Nutr.*, 56, 87-95.
12. Cunnane, S. C. (1990): *Biochim. Biophys. Acta*, 1036, 64-70.
13. Field, C. S., Angel, A. and Clandinin, M. T. (1985): *Am. J. Clin. Nutr.*, 42, 1206-1220.
14. Foreman-VanDrongelen, M. M. H. P., Hoswelingen, A. C. V., Kester, A. D. M., DeJong, A. E. P., Blanco, C. E., Hasaart, T. H. M. and Horstra, G. (1995): *Br. J. Nutr.*, 73, 405-422.
15. Hazel, J. R. and Williams, E. E. (1990): *Progr. Lip. Res.*, 29, 167-227.
16. Hudgins, L. L. and Hirsch J. (1991): *Am. J. Clin. Nutr.*, 53, 1372-1377.
17. Jones, P. J. H., Toy, B. R. and Cha, M. C. (1995): *J. Nutr.*, 125, 1175-1182.
18. Leaf, D. A., Connor, W. E., Barstad, L. and Sexton, G. (1995): *Am. J. Nutr.*, 62, 68-73.
19. Lebrazi, H., Chomard, P., Dumas, P. and Autissier, N. (1990): *Acta Endocrinol.*, 122, 379-384.
20. Lemonnier, D., Aubert, R., Suquet, J. P. and Rosselin, G. (1974): *Diabetologia*, 10, 697-701.
21. Leyton, J., Drury, P. J. and Crawford, M. A. (1987): *Br. J. Nutr.*, 57, 383-393.
22. Lin, D. S. and Connor, W. E. (1990): *Am. J. Clin. Nutr.*, 51, 535-539.
23. López-Soriano, F. J., Carbó, N. and Argiles, M. (1991): *Biochem. J.*, 274, 651-656.
24. MacDougal, D. E., Jones, P. J. H., Kitts, O. O. and Phang, T. T. (1996): *Am. J. Clin. Nutr.*, 63, 918-924.
25. Malcom, G. T., Bhattacharyya, A. K., Velez-Durán, M., Guzmán, M. A., Oalmann M. C. and Strong, J. P. (1989): *Am. J. Clin. Nutr.*, 52, 288-291.
26. Mersmann, H. J., McNeel, R. L., Morkeberg, J. C., Shparber, A. and Hachey, D. L. (1992): *J. Nutr.*, 122, 1952-1959.
27. Olsen, S., Hansen, H. S., Sandström, B. and Jensen, B. (1995): *Br. J. Nutr.*, 73, 387-395.
28. Phinney, S. D., Tang, A. B., Thurmond, D. C., Nakamura, T. and Stern, J. S. (1993): *Metabolism*, 42, 1127-1140.

29. Phinney, S. D., Tang, A. B., Routh, V., Stern, J. S. and Horwitz, A. (1994): *Int. J. Obes.*, 18 (Suppl. 2), 68.
30. Phinney, S. D., Stern, J. S., Burke, K. E., Tang, A. B., Miller, G. and Holman, T. (1994): *Am. J. Clin. Nutr.*, 60, 725- 729.
31. Popp-Snijders, L. and Blank, M. C. (1995): *Am. J. Clin. Nutr.*, 61, 360-365.
32. Raclot, T., Mioskowki, E., Bach, A. C and Grosolas, R. (1993): *J. Lipid Res.*, 34, 1515-1526.
33. Rossner, S., Walldins, G. and Bjorvell, H. (1989): *Int. J. Obes.*, 13, 603-612.
34. Shaw, M. J. and Hoch, F. L. (1976): *Life Sci.*, 19, 1359-1364.
35. Thorling, E. B. and Overvad, K. (1994): *Nutr. Res.*, 14, 569-576.
36. Whale, K. W. J. (1974): *Comp. Biochem. Physiol.*, 48B, 565-574.
37. Young, R. A., Fang, S. L., Presky, J. and Braverman, L. E. (1984): *Life Sci.*, 34, 1783-1790.