Influence of different dietary fats on triacylglycerol deposition in rat adipose tissue

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It has been demonstrated that triacylglycerol (TAG) mobilization from adipose tissue is selective and depends on fatty acid (FA) chain length, unsaturation and positional isomerism. The present study was performed to determine the influence of dietary fat on the composition of TAG stored in rat perirenal and subcutaneous adipose tissues. These results may provide information on the susceptibility of stored TAG to hydrolysis and further mobilization, and may help to establish an interrelationship between dietary composition and the FA efflux from adipose tissue. TAG molecular species and FA composition were determined by HPLC and GLC respectively. No significant differences were found in either FA or TAG composition between perirenal and subcutaneous adipose depots. The major FA in the dietary fats were present in the adipose tissues of the animals; in most cases, in similar proportions. However, differences were found between dietary and adipose tissue content of minor FA, which suggests that dietary FA composition is altered between ingestion and deposition in adipose tissue. The TAG molecular species of rat adipose tissue were enriched with the FA characteristic of each dietary fat. Dietary sunflower oil was responsible for enrichment with the most polar TAG. This finding may suggest easier mobilization of stored TAG. In conclusion, the process of fatty acid and TAG deposition in rat adipose tissue is selective, and depends on the composition of the diet.

Triacylglycerol: Adipose tissue: Dietary fat

Adipose tissue accumulates triacyglycerols (TAG) during periods of overfeeding, and releases free fatty acids (FA) during starvation and between meals. Once FA enter the bloodstream, they are involved in several important functions, such as the turnover of cell-membrane lipids or the synthesis of lipid mediators (e.g. eicosanoids; Crawford, 1983). In spite of the importance of the dynamic state of TAG storage and fatty acid release in obesity and its related pathologies, these processes are still incompletely understood.

The deposition and the mobilization of TAG may be influenced by dietary fat. In this context, the influence of dietary FA on the FA profile of membrane adipocytes and its consequences on lipid mobilization have been widely studied. In a previous study performed in our laboratory (Portillo *et al.* 1999), using the same experimental design as

the present study, we found that phospholipids in membrane adipocytes from subcutaneous and perirenal adipose tissues paralleled, to some extent, dietary FA profiles. Some of the changes induced by the dietary lipid source had an effect on lipolytic activity at the β-adrenoceptor level, but no effects were found at subsequent levels in the adenylate cyclase cascade.

Dietary fat composition may not only influence the FA profile of membrane phospholipids, but also the composition of stored TAG (Ruiz-Gutiérrez *et al.* 1992). FA deposition in adipose tissue bears some resemblance to the FA composition of the diet (Thomas *et al.* 1981). Nevertheless, differences between dietary lipids and adipose tissue exist, probably due to intermediate processes between fat ingestion and adipose tissue deposition. It has been demonstrated that endogenous FA are incorporated in

the formation of chylomicrons (Lambert *et al.* 1996). On the other hand, the rates of clearance of TAG during the postprandial period are different among the different molecular species (Botham *et al.* 1997; Abia *et al.* 1999) and FA are not released equally from circulating lipoproteins by lipoprotein lipase and transferred to adipose tissue (Wang *et al.* 1992).

With regard to lipid mobilization, the composition of the TAG stored in adipose tissue is an important factor affecting the release of FA into the bloodstream. Until recently, this process was widely considered to be random. However, it has now been demonstrated that this mobilization is selective and depends on FA chain length, degree of saturation and positional isomerism from adipose tissue (Gavino & Gavino, 1992; Raclot & Groscolas, 1995; Raclot *et al.* 1995; Raclot, 1997). These authors agree that the most easily mobilizable FA are $C_{16}-C_{20}$ with four or five double bonds, and that very-long-chain saturated and mono-unsaturated FA ($C_{20}-C_{24}$) are less mobilizable.

Raclot (1997) also asserts that the polarity of FA affects the hydrolysis process by hormone-sensitive lipase to a greater extent than the position in which these FA are attached. Thus, the accessibility of a TAG molecule to the lipase would be dependent on the polarity resulting from its molecular structure.

The aim of the present study was to determine the influence of different dietary fats on the composition of TAG stored in rat adipose tissue. In order to analyse the involvement of metabolic processes such as *de novo* FA synthesis in TAG storage, deposition index values were calculated. This index is defined as the relationship between the FA or TAG composition of the adipose tissue and their composition in the ingested fats. These data should provide interesting information which may help to predict the susceptibility to hydrolysis and further mobilization of TAG stored in adipose tissue from rats fed different lipid sources. A possible direct interrelationship between dietary composition and FA efflux from adipose tissue in interdigestion periods may be established. Since it has been widely reported that there are marked metabolic differences in lipid metabolism according to the adipose tissue site (Marchington & Pond, 1990; Richelsen *et al.* 1991; Calder *et al.* 1992; Belzung *et al.* 1993; Portillo *et al.* 2000), a comparison was also made between perirenal (PR) and subcutaneous (SC) adipose tissues.

Material and methods

Animals, diets and experimental design

Male Wistar rats were purchased from IFFA-Credo (Barcelona, Spain). They were individually housed in a room at $23 \pm 2°C$ and 50 % humidity, with a light–dark cycle of 12 h. Forty 7-week-old rats, weighing about 215 g, were randomly distributed into four groups of ten rats each. The animals were fed diets enriched in olive oil, beef tallow, sunflower oil or palm oil, which represented 40 % total energy ingested. The composition of the diets and the feeding protocol have been described previously (Portillo *et al.* 1999). The FA profiles and the TAG species in the dietary fats are shown in Tables 1 and 2. After 4 weeks rats

Table 1. Fatty acid composition of dietary fats (g/100 g total fatty acids)

Fatty acid	Olive oil	Beef tallow	Sunflower oil	Palm oil
14:0	0.06	4.62	0.07	1.30
16:0	11.88	29.50	6.35	43.94
18:0	2.67	25.45	1.61	4.25
20:0	0.06	0.13	nd	0.12
Total SFA	14.67	59.71	8.04	49.61
14:1n-5	nd	0.62	nd	0.05
16:1n-9	0.22	0.72	nd	0.05
16:1n-7	0.86	2.34	0.11	0.16
18:1n-9 *trans*	0.50	7.11	nd	0.16
18:1n-9	72.77	26.20	25.06	37.64
18:1n-7	4.40	1.59	1.56	1.69
20:1n-9	0.21	0.12	0.35	0.10
Total MUFA	78.97	38.71	27.08	39.85
18:2n-6	5.62	1.36	64.46	9.86
18:3n-3	0.74	0.22	0.42	0.58
Total PUFA	6.37	1.58	64.88	10.44

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected.

were killed by decapitation after an overnight fast. PR and SC adipose tissues were removed from the carcasses and weighed. They were immediately frozen in liquid N_2 and preserved at $-80°C$ until used.

Sample preparation

Total lipids were extracted from PR and SC adipose tissues following the method described by Folch *et al.* (1957). The lipid fractions were separated by TLC on silica gel 60 plates (Kieselgel 60 F_{254}; Merck), using an elution system of hexane–diethyl ether–acetic acid (80:20:1, by vol.;

Table 2. Composition of triacylglycerol (TAG) molecular species of dietary fats (g/100 g total TAG)

TAG	Olive oil	Beef tallow	Sunflower oil	Palm oil
LLLn	nd	nd	1.36	nd
OLnLn	nd	nd	0.04	nd
PLnLn	nd	nd	0.08	nd
LLL	0.01	0.01	8.21	0.10
OLLn	nd	nd	0.25	nd
SLnLn	nd	nd	1.54	nd
MLL	nd	nd	5.05	nd
MML	nd	nd	0.30	0.54
OLL(OOLn)	0.13	0.13	12.37	0.96
PLL(POLn)	0.41	0.21	41.75	3.88
PMM	0.19	0.16	0.67	1.07
OOL	2.24	1.60	2.92	2.31
POL(SLL)	9.62	4.03	22.55	34.54
PPL	0.12	1.75	0.33	1.40
OOO	65.60	11.52	0.18	2.79
POO	19.89	22.17	2.02	47.05
PPO	1.20	9.85	0.18	4.71
PPP	0.05	0.99	0.13	0.15
PMS	nd	5.57	nd	nd
SOO	0.34	18.87	0.04	0.23
POS	0.10	12.61	nd	0.25
SSO	nd	6.26	nd	nd
SSP	nd	4.27	nd	nd

M, myristic acid, 14:0; P, palmitic acid, 16:0; S, stearic acid, 18:0; O, oleic acid, 18:1; L, linoleic acid, 18:2; Ln, linolenic acid, 18:3; PPP, tripalmitoyl-glycerol; PMS, palmitoyl-myristoyl-stearoyl-glycerol; PLL, palmitoyl-dilinoleoyl-glycerol, nd, not detected.

Merck), according to the method of Ruiz-Gutiérrez *et al.* (1992). The TAG fraction was scraped off the silica, eluted with hexane–diethyl ether (9:1, v/v), passed through a stream of N_2 and stored below $-20°C$ until analysed.

Fatty acid analysis

TAG were transmethylated and the resulting fatty acid methyl esters analysed by GLC as described by Ruiz-Gutiérrez *et al.* (1992) using a model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame-ionization detector and a capillary silica column Supelcowax 10 (Sulpelco, Bellefonte, PA, USA) of 60 m length and 0·25 mm i.d.

The relationship between the FA composition of the adipose tissue and the composition of the fats ingested was used to calculate the FA deposition index for each FA. Only those FA found in the dietary fats were considered for this determination.

Triacylglycerol molecular species analysis

TAG, were evaporated to dryness under a vacuum, redissolved in *n*-hexane and passed through a filter with a pore size of 0·2 mm (Millipore, Bedford, MA, USA). The chromatographic system consisted of a model 2690 with a particle size of 3 μm (Phase Separations, Waters, Milford, MA, USA) and a column dead time of 1·99 min, according to the supplier. The mobile phase consisted of an initial elution gradient of 20 % (v/v) acetone in acetonitrile, with the percentage of acetone increasing to 45 % (v/v) at 4 min and finally to 80 % (v/v) at 40 min, which was maintained to the end of the analysis. The flow rate was 1·0 ml/min. The liquid chromatograph was coupled to a light-scattering detector model DDL31 (Eurosep Instruments, Cergy-Pontoise, France). The system was controlled by a computer through Millenium Software (Waters Co., Milford, MA, USA). Sigmoidal response curves were observed for all the standards determined. However, linear regression may also be valid (r^2 0·984–0·995) in the range of concentrations studied (between 0·25 and 2·5 mg/ml; Perona & Ruiz-Gutiérrez, 1999). Runs of 10 μl of a *n*-hexane solution containing pure TAG (Sigma grade, 99 % pure; Sigma, St Louis, MO, USA; 0·5 mg/ml): tritridecanoin, 1,3-dioleoyl-2-palmitoyl-glycerol, trimyristin, 1,3-dioleoyl-2-stearoyl-glycerol, 1,3-dioleoyl-2-linoleoyl-glycerol, tripentadecanoin, tripalmitin, triolein and trilinolein) were injected in quintuplicate in order to establish the capacity factor of the system. All the solvents used, both in dissolving sample TAG and in the mobile phase, were HPLC grade (Merck). The TAG composition was predicted by means of the relationship between the capacity factor and molecular variables of pure triacylglycerols.

The values of the equivalent C number (ECN) were calculated as $ECN = CN - 2ND - 2NUFA$, where CN is the number of C atoms, ND the number of double bonds and NUFA the number of unsaturated FA attached to the glycerol molecule. A linear regression analysis was applied to relate the ECN to the log of the capacity factor for the pure TAG. The TAG composition of the samples was calculated by comparison of the ECN of the chromatographic peaks and the theoretical ECN of all possible TAG. These data were calculated by combination of FA obtained in the GLC analysis in groups of three. We considered all the stereospecific positions in the glycerol backbone as equivalents, since HPLC cannot separate positional isomers (Perona *et al.* 1998a; Perona & Ruiz-Gutiérrez, 1998). TAG were quantified using tridecanoin as internal standard. The method was adapted from Nájera *et al.* (1998).

The deposition indexes, calculated as the relationship between the TAG composition of the adipose tissue and the composition of the fats ingested for each TAG molecular species, were plotted *v.* ECN of each TAG, which was taken as an index of polarity, since TAG molecules elute from the HPLC column in increasing ECN in a reversed-phase system. Thus, the lowest ECN values correspond to the most polar molecules and the highest to the most apolar ones.

Statistical analysis

The significance of the differences among the dietary groups and between anatomical sites of adipose tissue was assessed by one-way ANOVA with unpaired Student's *t* test for multiple comparisons. Pearson correlation coefficients were calculated to examine the relationship between TAG deposition index and ECN. All comparisons were considered statistically significant at $P < 0.05$. The analyses were done with the GraphPAD InStat (GraphPAD Software, San Diego, CA, USA) and CoStat (CoHort Software, Minneapolis, MN, USA).

Results

Animals fed diets enriched with different fats showed a similar weight gain during the experimental period. The weight of adipose tissue from each anatomical site was also similar in all the groups (Portillo *et al.* 1999).

The FA composition of the TAG stored in adipose tissues is shown in Tables 3 (PR) and 4 (SC). Significant differences were found among rats fed the different experimental diets. The content of oleic acid (18:1*n*-9) was higher in the adipose tissue of rats fed olive oil (59·73 g/100 g total FA for PR and 57·22 g/100 g total FA for SC). This finding was reflected in the global content of monounsaturated FA, which accounted for approximately 70 % of the total FA. Relatively high amounts of palmitic (16:0; 16·67 g/100 g total FA for PR and 16·71 g/100 g total FA for SC) and linoleic (18:2*n*-6; 10·60 g/100 g total FA for PR and 13·06 g/100 g total FA for SC) acids were found in the adipose tissues of rats fed olive oil. The adipose tissue of rats fed beef tallow showed a greater accumulation of saturated FA, than that of rats fed the other diets. Myristic acid (14:0) accounted for 2·69 g/100 g total FA in PR and 3·35 g/100 g total FA in SC, palmitic acid (16:0) reached approximately 30 g/100 g total FA in both sites and stearic acid (18:0) was detected at levels of 7 g/100 g total FA for PR and 8 g/100 g total FA for SC. The content of elaidic acid (18:1*n*-9 *trans*, 2·87 g/100 g total FA for PR and 2·93 g/100 g total FA for SC) was also higher in the adipose tissue of rats fed beef tallow. The

Table 3. Fatty acid composition (g/100 g total fatty acids) of perirenal adipose tissue from rats fed the experimental diets*

(Values expressed as means and standard deviations)

Fatty acids	Olive oil		Beef tallow		Sunflower oil		Palm oil	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0·76[a]	0·06	2·69[b]	0·31	0·94[a]	0·13	1·26[a]	0·21
16:0	16·67[a]	0·85	25·38[b]	0·72	16·45[a]	1·40	29·32[c]	0·54
18:0	2·46[a]	0·15	7·07[b]	0·53	3·21[a]	0·29	2·95[a]	0·24
20:0	0·29	0·01	0·21	0·02	0·05	0·02	0·03	0·01
22:0	0·08	0·02	0·08	0·02	nd	nd	0·06	0·01
Total SFA	20·02[a]	1·03	35·39[b]	1·09	21·08[a]	0·94	33·74[b]	0·53
14:1n-5	0·18	0·09	0·33	0·12	0·15	0·01	0·15	0·03
16:1n-9	0·46	0·06	0·53	0·04	0·25	0·06	0·34	0·04
16:1n-7	2·42[a]	0·40	5·23[b]	0·48	2·98[a]	0·97	3·19[a]	0·81
18:1n-9 trans	0·03[a]	0·00	2·87[b]	0·31	0·08[a]	0·02	0·16[a]	0·06
18:1n-9	59·73[a]	1·71	41·40[b]	1·36	26·51[c]	0·64	45·17[b]	2·30
18:1n-7	4·74[a]	0·39	4·65[a]	0·20	2·34[b]	0·33	3·38[b]	0·15
20:1n-9	0·14	0·02	0·21	0·02	0·16	0·02	0·23	0·03
20:1n-7	nd	nd	nd	nd	0·21	0·03	0·19	0·03
24:1n-9	nd	nd	nd	nd	0·06	0·02	0·20	0·03
Total MUFA	67·99[a]	1·29	55·49[b]	1·75	32·46[c]	1·77	52·62[b]	2·15
18:2n-6	10·60[a]	1·03	7·91[a]	1·70	45·04[b]	2·84	12·77[a]	2·14
20:2n-6	0·04	0·01	nd	nd	0·22	0·06	0·03	0·04
20:3n-6	0·10	0·02	0·09	0·03	0·09	0·05	0·06	0·01
20:4n-6	0·19	0·05	nd	nd	0·61	0·30	0·15	0·03
22:5n-6	nd	nd	nd	nd	0·42	0·31	0·02	0·01
Total PUFA n-6	10·93[a]	1·09	8·00[a]	1·68	46·38[b]	2·38	13·03[a]	2·21
18:3n-3	0·63	0·27	0·74	0·13	0·41	0·07	0·30	0·04
22:6n-3	0·02	0·01	0·12	0·05	nd	nd	nd	nd
Total PUFA n-3	0·65	0·28	0·86	0·15	0·41	0·07	0·30	0·04

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, *t* test).
SFA, saturated fatty acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected.
* For details of fatty acid and triacylglycerol composition of dietary fats, see Tables 1 and 2, and for details of experimental procedures, see p. 766.

Table 4. Fatty acid composition (g/100 g total fatty acids) of subcutaneous adipose tissue from rats fed the experimental diets*

(Values expressed as means and standard deviations)

Fatty acids	Olive oil		Beef tallow		Sunflower oil		Palm oil	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0·91[a]	0·14	3·35[b]	0·19	1·05[a]	0·09	1·21[a]	0·23
16:0	16·71[a]	1·65	29·49[b]	0·58	16·12[a]	2·19	26·57[b]	1·47
18:0	2·50[a]	0·20	8·27[b]	0·05	3·38[a]	0·07	3·24[a]	0·44
20:0	0·05[a]	0·01	0·25[b]	0·02	0·04[a]	0·00	0·03[a]	0·00
22:0	nd	nd	0·41	0·15	0·05	0·01	0·03	0·00
Total SFA	20·14[a]	1·56	40·89[b]	0·67	20·58[a]	2·26	31·05[c]	1·32
14:1n-5	0·15	0·02	0·67	0·02	0·14	0·03	0·19	0·02
16:1n-9	0·58	0·02	0·50	0·08	0·28	0·02	0·36	0·04
16:1n-7	2·83	0·83	3·86	0·07	2·47	0·46	2·06	0·02
18:1n-9 trans	0·12[a]	0·05	3·26[b]	0·08	0·09[a]	0·04	0·11[a]	0·02
18:1n-9	57·22[a]	3·90	33·93[b]	0·91	26·55[b]	1·13	45·10[c]	1·54
18:1n-7	4·24[a]	0·21	3·90[a]	0·23	2·21[b]	0·18	2·82[b]	0·40
20:1n-9	0·30	0·04	0·38	0·02	0·19	0·02	0·20	0·03
20:1n-7	0·18	0·04	nd	nd	0·30	0·03	0·21	0·04
24:1n-9	0·09	0·06	0·08	0·12	nd	nd	0·11	0·20
Total MUFA	65·71[a]	2·93	45·49[b]	0·22	32·23[c]	1·45	50·39[b]	1·97
18:2n-6	13·06[a]	1·17	10·23[a]	0·21	44·47[b]	3·92	16·04[a]	2·28
20:2n-6	0·08	0·03	nd	nd	0·20	0·06	0·15	0·13
20:3n-6	0·06	0·05	nd	nd	0·14	0·04	0·08	0·06
20:4n-6	0·13	0·09	nd	nd	0·73	0·35	0·21	0·04
22:5n-6	0·16	0·12	0·05	0·11	0·20	0·09	0·13	0·14
Total PUFAn-6	13·49[a]	1·21	10·28[a]	0·21	45·74[b]	4·03	16·61[a]	2·36
18:3n-3	0·60	0·13	0·73	0·05	0·35	0·16	0·51	0·02
22:6n-3	nd	nd	nd	nd	0·05	0·02	nd	nd
Total PUFAn-3	0·60	0·13	0·73	0·05	0·40	0·18	0·51	0·02

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, *t* test).
SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected.
* For details of fatty acid and triacylglycerol composition and dietary fats, see Tables 1 and 2, and for details of experimental procedures, see p. 766.

Table 5. Composition of triacylglycerol (TAG) molecular species (g/100 g total TAG) of perirenal adipose tissue from rats fed the experimental diets*

(Values expressed as means and standard deviations)

TAG	Olive oil Mean	SD	Beef tallow Mean	SD	Sunflower oil Mean	SD	Palm oil Mean	SD
LLLn	nd	nd	nd	nd	0·03	0·01	nd	nd
OLnLn	nd	nd	nd	nd	0·02	0·01	nd	nd
PLnLn	nd	nd	nd	nd	nd	nd	nd	nd
MMLn	nd	nd	nd	nd	0·04	0·02	nd	nd
LLL	0·05[a]	0·03	0·04[a]	0·01	10·58[b]	1·14	0·04[a]	0·02
OLLn	0·05	0·02	0·03	0·01	0·25	0·03	0·02	0·01
SLnLn	0·02	0·00	0·05	0·02	0·08	0·01	0·02	0·01
PLLn	0·03	0·02	nd	nd	0·09	0·08	0·02	0·02
MLL	0·01	0·01	0·02	0·01	0·05	0·05	0·02	0·01
MML	0·02	0·01	0·02	0·02	0·02	0·01	0·02	0·01
OLL(OOLn)	1·58[a]	0·61	0·44[a]	0·31	17·73[b]	4·17	1·34[a]	0·84
PLL(POLn)	1·84[a]	0·91	1·17[a]	0·40	16·36[b]	2·82	3·58[a]	1·41
PPLn	0·08	0·05	0·38	0·12	0·28	0·14	0·17	0·04
LPM	0·08	0·03	0·30	0·12	0·34	0·28	0·17	0·07
OMM	0·04	0·03	0·19	0·14	0·06	0·01	0·02	0·01
PMM	0·02[a]	0·02	nd	nd	nd	nd	0·01[a]	0·01
OOL	17·80[a]	1·76	4·40[b]	1·02	11·96[a]	3·05	12·56[a]	2·91
POL(SLL)	14·79	2·81	13·91	2·44	21·35	3·64	19·46	3·01
PPL	1·52[a]	0·60	1·74[a]	0·43	4·78[b]	1·66	6·39[b]	2·84
PSLn	nd	nd	2·98[a]	0·93	0·07[b]	0·01	0·06[b]	0·06
PPPo	0·11	0·05	0·83	0·26	0·18	0·08	0·13	0·04
OOO	25·26[a]	6·15	10·23[b]	0·62	2·55[c]	1·65	12·81[b]	1·54
POO	24·58[a]	4·97	37·58[b]	3·06	9·40[c]	4·50	22·98[a]	4·39
PPO	4·86[a]	0·80	16·28[b]	2·91	2·86[a]	0·43	16·88[b]	1·84
PPP	0·15[a]	0·03	1·51[b]	0·46	0·49[a]	0·60	1·02[b]	0·27
SOO	0·12	0·06	0·33	0·07	nd	nd	0·05	0·02
POS	2·08[a]	0·54	3·98[b]	0·47	0·17[c]	0·02	1·06[a]	0·08
SSO	0·47[a]	0·19	3·63[b]	0·70	0·31[a]	0·21	1·17[a]	0·08

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, t test).

M, myristic acid, 14:0; P, palmitic acid, 16:0; S, stearic acid, 18:0; Po, palmitoleic, 16:1; O, oleic acid, 18:1; L, linoleic acid, 18:2; Ln, linolenic acid, 18:3; PPP, tripalmitoyl-glycerol; PSLn, palmitoyl-stearoyl-linolenoyl-glycerol; PLL, palmitoyl-dilinoleoyl-glycerol; nd, not detected.

* For details of fatty acid and triacylglycerol composition of dietary fat, see Tables 1 and 2, and for details of experimental procedures, see p. 766.

adipose tissue of rats fed sunflower oil was characterized by a high content of linoleic acid (45·04 g/100 g total FA for PR and 44·47 g/100 g total FA for SC) and derivatives of the *n*-6 family, with a concomitant decrease in monounsaturated and saturated FA. Palmitic and oleic acids accounted for approximately 70 g/100 g total FA in the adipose tissue of rats fed palm oil.

Tables 5 and 6 show the TAG molecular species composition of rat PR and SC adipose tissues respectively. An HPLC chromatogram of adipose tissue TAG species profile is shown in Fig. 1. Oleic acid-containing TAG were significantly enhanced in the adipose tissue of rats fed olive oil. Triolein (25·26 g/total TAG for PR and 25·84 g/100 g total TAG for SC) and dioleoyl-linoleoyl-glycerol (17·80 g/100 g total TAG for PR and 18·53 g/100 g total TAG for SC) showed higher concentrations than in rats fed the other dietary fats. As expected, the adipose tissue of rats fed beef tallow was enriched in those TAG formed with palmitic and stearic acids (palmitoyl-dioleoyl-glycerol, dipalmitoyl-oleoyl-glycerol, palmitoyl-oleoyl-stearoyl-glycerol and distearoyl-oleoyl-glycerol). In addition, a high amount of palmitoyl-stearoyl-linolenoyl-glycerol was found (2·98 g/100 g total TAG for PR and 4·67 g/100 g total TAG for SC) compared with rats in the other dietary groups, in which only traces were found. Sunflower oil was responsible for an elevation of the content of linoleic acid-containing species (trilinolein, oleoyl-dilinoleoyl-gly-

cerol and palmitoyl-dilinoleoyl-glycerol). When palm oil was administered to rats, the adipose tissue showed higher contents of palmitoyl species (dipalmitoyl-linoleoyl-glycerol and dipalmitoyl-oleoyl-glycerol). The TAG molecule comprising the three main FA present in the dietary fats studied, i.e. palmitoyl-oleoyl-linoleoyl-glycerol, was not found in significantly different amounts in the adipose tissue from the different dietary groups.

When PR and SC adipose tissues were compared, slight differences were only observed in the rats fed beef tallow. In this group, the content of oleic acid (18:1*n*-9) was significantly higher ($P < 0.05$) in PR than in SC. With regard to TAG molecular species composition, a significantly higher amount of palmitoyl-dioleoyl-glycerol ($P < 0.05$) and a significantly lower amount of oleoyl-dilinoleoyl-glycerol and palmitoyl-dilinoleoyl-glycerol ($P < 0.05$) were found in PR. For clarity, these data are not shown in Tables 3–6.

Values for the deposition index for FA from dietary fats are shown in Table 7. Rats fed olive oil showed a greater deposition of linoleic acid at both sites of adipose tissue (1·89 for PR and 2·32 for SC), compared with the other main FA present in olive oil. However, this effect was more evident in rats fed beef tallow, with deposition indexes of 5·80 and 7·28 for PR and SC adipose tissues respectively, although this FA was less prevalent in beef tallow than in olive oil. On the contrary, stearic acid was deposited at very

Table 6. Composition of triacylglycerol (TAG) molecular species (g/100 g total TAG) of subcutaneous adipose tissue from rats fed the experimental diets[*]

(Values expressed as means and standard deviations)

TAG	Olive oil Mean	SD	Beef tallow Mean	SD	Sunflower oil Mean	SD	Palm oil Mean	SD
LLLn	0·02	0·01	nd	nd	0·03	0·01	0·01	0·01
OLnLn	nd	nd	0·02	0·01	0·02	0·01	0·01	0·00
PLnLn	nd	nd	0·03	0·02	0·09	0·06	0·03	0·03
LLL	0·16[a]	0·11	0·11[a]	0·07	9·80[b]	3·14	0·20[a]	0·04
OLLn	0·08	0·06	0·05	0·02	0·27	0·08	0·11	0·08
SLnLn	0·03	0·02	0·07	0·01	0·23	0·14	0·12	0·08
PLLn	0·06	0·05	0·08	0·05	0·23	0·11	0·16	0·08
MLL	0·02	0·01	0·05	0·04	0·24	0·09	0·04	0·02
MML	nd	nd	0·07	0·05	0·05	0·02	0·06	0·04
OLL(OOL)	2·33[a]	1·42	1·78[a]	0·39	19·48[b]	4·04	2·86[a]	1·33
PLL(POLn)	3·61[a]	1·10	3·34[a]	1·03	16·23[b]	1·98	3·91[a]	2·05
PPLn	0·45	0·05	nd	nd	0·33	0·10	0·29	0·11
LPM	0·16	0·08	0·40	0·16	nd	nd	nd	nd
OMM	0·06	0·04	0·57	0·33	0·10	0·04	0·05	0·03
PMM	0·03	0·02	nd	nd	nd	nd	0·06	0·04
OOL	18·53[a]	1·28	4·10[b]	0·70	13·69[c]	1·10	13·74[c]	1·37
POL(SLL)	15·46	2·68	21·91	2·76	20·86	2·69	20·35	2·51
PPL	1·69[a]	0·72	3·53[b]	0·33	3·31[b]	0·94	3·56[b]	1·12
PSLn	nd	nd	4·67[a]	1·43	0·03[b]	0·02	nd	nd
PPPo	0·13	0·05	1·14	0·40	0·16	0·07	0·16	0·12
OOO	25·84[a]	7·25	10·23[b]	1·73	1·63[c]	0·37	11·34[b]	2·30
POO	23·89[a]	4·30	21·53[a]	1·89	6·28[b]	0·74	22·75[a]	5·01
PPO	4·75[a]	1·76	14·86[b]	3·14	5·46[a]	3·97	15·49[b]	1·78
PPP	0·17[a]	0·07	1·63[b]	0·42	0·95[a]	0·48	1·12[b]	0·42
SOO	0·15	0·09	0·31	0·09	0·08	0·04	0·11	0·04
POS	1·72[a]	0·35	4·27[b]	0·65	0·27[c]	0·01	1·69[a]	0·46
SSO	0·68[a]	0·29	4·24[b]	0·18	0·20[a]	0·09	1·78[c]	0·64

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, *t* test).

M, myristic acid, 14:0; P, palmitic acid, 16:0; S, stearic acid, 18:0; Po, palmitoleic, 16:1; O, Oleic acid, 18:1; L, linoleic acid, 18:2; Ln, linolenic acid, 18:3; PPP, tripalmitoyl-glycerol; PSLn, palmitoyl-stearoyl-linolenoyl-glycerol; PLL, palmitoyl-dilinoleoyl-glycerol; nd, not detected.

[*] For details of fatty acids and triacylglycerol composition of dietary fat, see Tables 1 and 2, and for details of experimental procedures, see p. 766.

low levels in adipose tissue of rats fed beef tallow (0·28 for PR and 0·32 for SC). The main saturated FA (palmitic and stearic acids) had higher deposition index values (>2) for both adipose tissue sites in rats fed either sunflower oil or olive oil, but not in rats fed palm oil. The deposition index of oleic acid, one of the main dietary FA present in palm oil, was approximately 1 in rats fed palm oil.

The deposition index of TAG molecular species was also calculated and is represented in Fig. 2. Olive oil (Fig. 2(a))

and beef tallow (Fig. 2(b)) caused an accumulation of the most polar TAG (ECN 41·4–45·8) in adipose tissue, in relation to the content in the dietary fats. In contrast, the content of some of the most apolar TAG (high ECN, triolein and stearoyl-dioleoyl-glycerol) was even lower than that of the diet. This fact was reflected in a negative tendency, with a decreased storage of TAG with high ECN (olive oil: $y = -1.25 \times +62.13$, r 0·801, $P = 0.003$ for PR and $y = -1.92x + 95.54$, r 0·775, $P = 0.0051$ for SC; beef

Table 7. Deposition index of fatty acids in perirenal (PR) and subcutaneous (SC) adipose tissues from rats fed the experimental diets*

Fatty acids	Olive oil PR	SC	Beef tallow PR	SC	Sunflower oil PR	SC	Palm oil PR	SC
14:0	12·63	15·08	0·58	0·72	12·98	14·39	0·97	0·93
14:1*n*-5	nd	nd	0·53	0·96	nd	nd	3·07	3·75
16:0	1·40	1·41	0·86	0·99	2·59	2·54	0·67	0·60
16:1*n*-9	2·07	2·62	0·73	0·69	nd	nd	6·89	7·27
16:1*n*-7	2·83	3·31	2·24	1·57	26·09	21·57	19·94	12·84
18:0	0·92	0·94	0·28	0·32	1·99	2·10	0·69	0·76
18:1*n*-9 *trans*	0·05	0·24	0·40	0·41	nd	nd	0·97	0·70
18:1*n*-9	0·82	0·79	1·58	1·28	1·06	1·06	1·20	1·20
18:1*n*-7	1·08	0·96	2·92	2·44	1·50	1·42	2·00	1·67
18:2*n*-6	1·89	2·32	5·80	7·28	0·70	0·69	1·30	1·63
18:3*n*-3	0·85	0·81	3·36	3·32	0·98	0·83	0·51	0·88
20:0	4·78	0·77	1·58	1·89	nd	nd	0·25	0·27
20:1*n*-9	0·65	1·41	1·71	2·30	0·44	0·53	2·28	2·01

nd, not detected.

* For details of fatty acid and triacylglycerol composition of dietary fat, see Tables 1 and 2, and for details of experimental procedures, see p. 766.
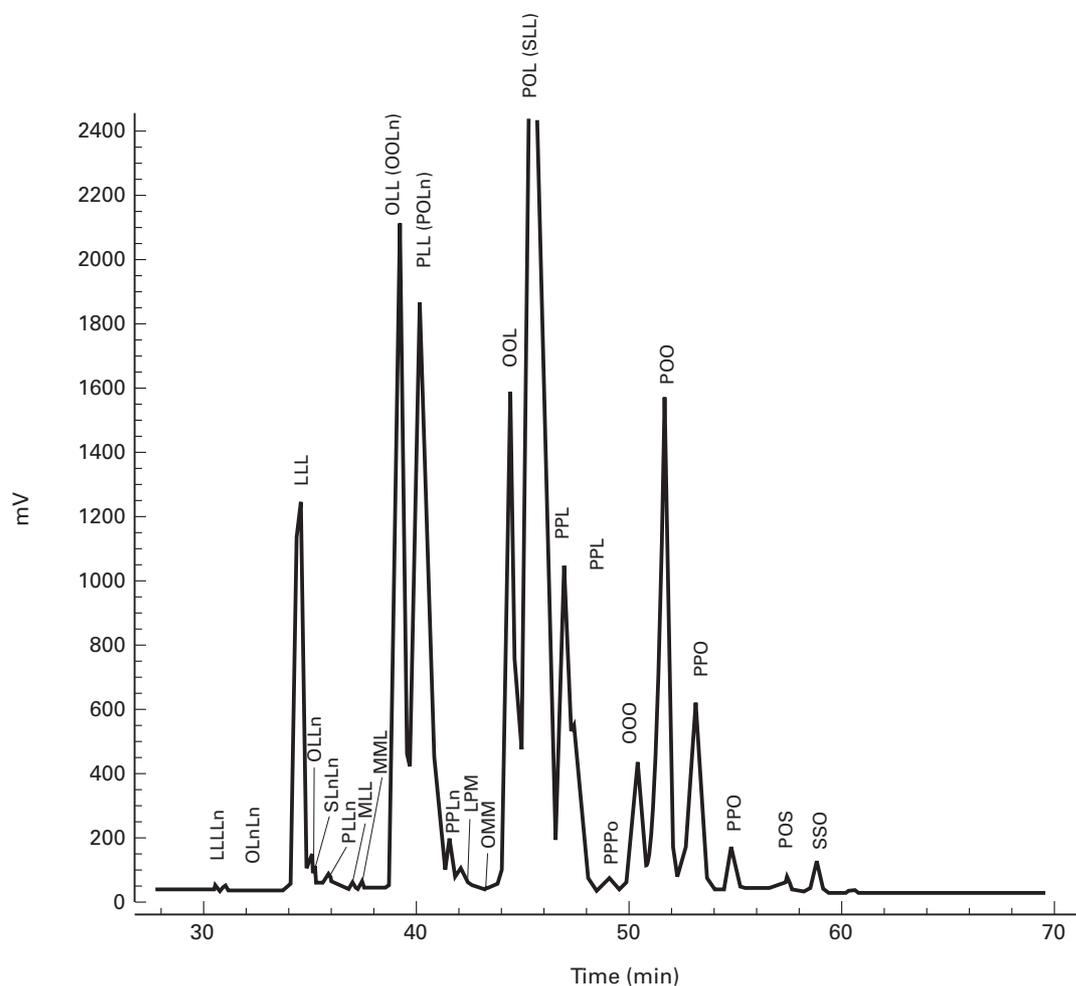
Fig. 1. Triacylglycerol (TAG) molecular species chromatogram of rat adipose tissue by HPLC. For details of elution conditions, see p. 767. M, myristic acid, 14:0; P, palmitic acid, 16:0; S, stearic acid, 18:0; O, oleic acid, 18:1; L, linoleic acid, 18:2; linolenic acid, 18:3; PPP, tripalmitoyl-glycerol; LPM, linoleoyl-palmitoyl-myristoyl-glycerol; PLL, palmitoyl-dilinoleoyl-glycerol.

tallow: $y = -0.54x + 26.88$, r 0.788, $P = 0.004$ for PR and $y = -1.95x + 95.28$, r 0.837, $P = 0.0013$ for SC). Such an effect was not observed for sunflower oil (Fig. 2(c)) or palm oil (Fig. 2(d)) (sunflower oil: $y = 1.65x - 68.93$, r 0.234, $P = 0.1198$ for PR and $y = 1.44x - 59.58$, r 0.210, $P = 0.2658$ for SC; palm oil: $y = 0.22x - 7.54$, r 0.276, $P = 0.4122$ for PR and $y = 0.18x - 5.41$, r 0.126, $P = 0.5269$ for SC), although sunflower oil was responsible for the specific deposition of triolein, dipalmitoyl-oleoyl-glycerol and dipalmitoyl-linoleoyl-glycerol.

Discussion

The present work was performed in order to study the deposition of dietary TAG from different origins (olive oil, beef tallow, sunflower oil or palm oil) in rat adipose tissues. It is well known that during high-fat feeding the FA composition of TAG in adipose tissue largely reflects that of the diet (Lin & Connor, 1990). A similar finding was also obtained in the present study. The main FA of the dietary fats were present in the adipose tissue of the animals, in most cases in similar proportions. However,

differences were found between the dietary and adipose tissue contents of minor fatty acids, which suggests that dietary FA composition is altered between ingestion and deposition in adipose tissue. It has been suggested that when a diet provides a high amount of a specific FA, a restructuring and synthesis of TAG takes place to protect the tissues from sudden changes (Fielding et al. 1996; Lambert et al. 1996). This process has been observed to start in the intestine. This effect was shown when the values of FA deposition index were calculated for each dietary fat (Table 7). High values for deposition index were obtained for the minor FA. The myristic acid content (14:0) was increased more than 10-fold in the adipose tissue of rats fed olive oil or sunflower oil compared with the dietary fats, suggesting de novo synthesis. Similarly, palmitoleic acid (16:1n-7) content was raised in the adipose tissue of rats fed sunflower oil or palm oil, with values for deposition index of about 20, suggesting an increase in the desaturation of palmitic acid. Lin et al. (1993) reported that, in general terms, the FA composition of dietary lipids is conserved in the adipose tissue of rabbits. However, despite using the deposition index, they did not calculate values for minor
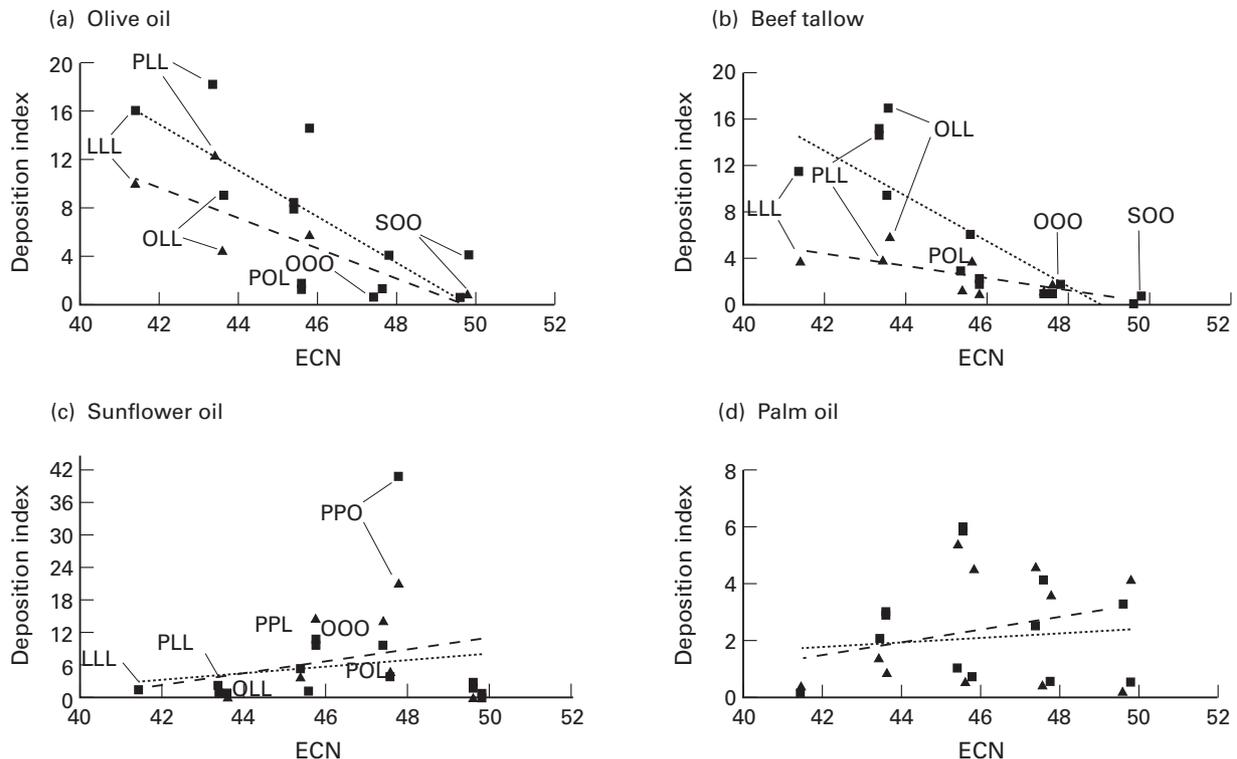
Fig. 2. Deposition index *v.* the equivalent carbon number (ECN) of triacylglycerols in perirenal (PR; ▲– – –▲) and subcutaneous (SC; ■·····■) adipose tissues from rats fed (a) olive oil, (b) beef tallow, (c) sunflower oil and (d) palm oil. The deposition index (the relationship between triacylglycerol composition of adipose tissue and that of dietary fat) and ECN (an index of polarity) were calculated as described on p. 769. LLL, trilinolein; PLL, palmitoyl-dilinoleoyl-glycerol; OLL, oleoyl-dilinoleoyl-glycerol; PPL, dipalmitoyl-linoleoyl-glycerol; OOO, triolein; SOO, stearoyl-dioleoyl-glycerol; PPO, dipalmitoyl-oleoyl-glycerol; POL, palmitoyl-oleoyl-linoleoyl-glycerol. (A) PR *r* 0·801, SC *r* 0·775; (B) PR *r* 0·788, SC *r* 0·837; (C) PR *r* 0·234, SC *r* 0·210; (D) PR *r* 0·276, SC *r* 0·126.

fatty acids, which show the greatest differences in the present study.

The composition of adipose tissue affects the release of FA into the bloodstream. It has been suggested that the release process is selective, based on the polarity of the stored FA rather than on the specificity of hormone-sensitive lipase; i.e., the more polar a FA (low number of C atoms or high extent of unsaturation), the easier the access to hormone-sensitive lipase and thus, its hydrolysation (Gavino & Gavino, 1992; Connor *et al.* 1996; Raclot, 1997). This relationship would imply that, depending on their polarity, FA would be more or less rapidly released. However, it is evident that as FA are stored as TAG molecules in adipose tissue, their accessibility will be dependent on the molecular polarity of the TAG.

The TAG molecular species in the rat adipose tissue (Tables 5 and 6) were enriched with the FA characteristic of each dietary fat source. Palm oil and beef tallow were responsible for the accumulation of TAG containing saturated FA. As expected, we found important amounts of stearic acid-containing TAG in the adipose tissue of rats fed beef tallow, but we were surprised by the high content in dioleoyl-linoleoyl-glycerol observed in the adipose depot of rats consuming the palm-oil-enriched diet. This TAG was also found in notable amounts in the adipose tissue of rats fed olive oil or sunflower oil. Actually, the deposition index was about 4·5 in rats fed sunflower oil, about 5·5 in

rats fed palm oil and about 8·0 in rats fed olive oil. It seems that there is a need to accumulate dioleoyl-lindeoyl-glycerol in rat adipose tissue. Sunflower oil was responsible for adipose tissue enrichment with the most polar TAG (trilinolein, dilinoleoyl-oleoyl-glycerol and dilinoleoyl-palmitoyl-glycerol). This finding is in good agreement with those of Huang *et al.* (1992) in rat adipose tissue; they reported that a diet highly enriched with linoleic acid may cause linoleic acid to replace other FA in the TAG molecules, increasing the content of dilinoleoyl-acyl-glycerol species. In olive oil, the content of dilinoleoyl species was very low, while dioleoyl species accounted for about 70 g/100 g total TAG. Recently, we have found similar amounts in the liver of rats fed olive oil (Perona & Ruiz-Gutiérrez, 1998; Perona *et al.* 1998b). Thus, not only the TAG composition of adipose tissue but also that of other organs might be modified via the diet, with evident physiological implications (Chen & Cunnane, 1992, 1993; Chen *et al.* 1992). It is very likely that TAG containing long-chain polyunsaturated FA were present in adipose tissues, but our chromatographic system was not able to detect them. (These TAG only account for 2 g/100 g total TAG.)

When we plotted the deposition index values for some of the main TAG *v.* their corresponding ECN, we found important differences in the effects of diet. While rats fed diets enriched with olive oil or beef tallow showed a

tendency to accumulate polar TAG (low ECN), neither this nor the tendency to accumulate apolar TAG (high ECN) was observed in rats fed sunflower oil or palm oil. The accumulation of TAG in response to a diet enriched with palm oil showed comparatively lower deposition index values for the TAG species studied. In contrast, some TAG were specifically accumulated in the adipose tissue of rats fed sunflower oil, in relation to their content in the oil. Despite the fact that linoleic-acid-containing TAG are in the majority in the adipose tissue of these rats, the deposition index values for dipalmitoyl-linoleoyl-glycerol, dipalmitoyl-oleoyl-glycerol and triolein were >10. We must emphasize that these TAG are minor TAG in sunflower oil (<1 g/100 g total TAG). Thus, it seems that there is a need to accumulate these TAG in rat adipose tissue when a diet containing relatively polar TAG is fed. The increase in saturated FA in the TAG species of rat adipose tissue might be related to a requirement for these FA for metabolic functions leaving linoleic acid for other physiological functions. In addition, linoleic acid-containing TAG (trilinolein, oleoyl-dilinoleoyl-glycerol, palmitoyl-dilinoleoyl-glycerol and palmitoyl-oleoyl-linoleoyl-glycerol) showed low deposition index values in the adipose tissue of rats fed a diet rich in this FA. This factor suggests that an excess of linoleic acid is diverted towards other physiological functions. However, when the diet is low in linoleic acid (olive oil and beef tallow), the animal tends to accumulate TAG containing linoleic acid, which are oxidized more slowly. This finding is in agreement with the work of Chen & Cunnane (1991), who reported an increase in these TAG in fasting rats.

In general, similar FA profiles and TAG compositions were observed in adipose tissue from both anatomical sites, in all groups. Slight differences between PR and SC depots were observed only in rats fed beef tallow.

In summary, we have found that the process of FA and TAG deposition in rat adipose tissue is selective, and depends on the composition of the diet. This finding has important implications for obesity and its related pathologies, since the depletion of lipids stored in adipose tissue by TAG hydrolysis is related to the composition of the tissue. Mobilization of TAG stored in the adipose tissues from rats fed diets enriched with sunflower oil may be related to the high content of the most-polar TAG species (trilinolein, dilinoleoyl-oleoyl glycerol and dioleoyl-palmitoyl-glycerol). We believe that it is not sufficient to determine the FA composition in the study of TAG hydrolysis from adipose tissue: the composition of the TAG molecular species must also be considered, as the molecular polarity of TAG seems to be a determinant in the process of FA release.

Acknowledgements

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