

Monounsaturated *n*-9 fatty acids and adipocyte lipolysis in rats

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To investigate the role of the monounsaturated *n*-9 fatty acids (MUFA) in the lipolytic activity of adipocytes, a study was carried out in which an increase in MUFA was produced in the tissues by two different methods; by the dietary enrichment of oleic acid or by producing an essential fatty acid deficiency syndrome. For this, forty-five male Sprague–Dawley rats were fed with a normal-energy diet and were subdivided into three groups. The diets varied in the type of dietary fat; palmitic acid, olive oil, or soyabean oil + palmitic acid. At the end of the study measurements were taken of weight, plasma leptin, tissue concentration of fatty acids, fat-cell size in the epididymal and the omental adipose tissues, adipocyte lipolytic activity of both tissues after stimulation with adrenaline, and the capacity of insulin to inhibit lipolysis. The baseline and adrenaline-stimulated lipolytic activity were greater and the anti-lipolytic capacity of insulin lower in the animals undergoing an increase in MUFA in the tissues (palmitic-acid and olive-oil diets). The area under the curve of glycerol, used as an indicator of lipolytic activity, was positively correlated with the concentration of MUFA and negatively with polyunsaturated fatty acids in the adipose tissues. It is concluded that an increase in tissue MUFA, however obtained, induces an increase in lipolytic activity.

Adipocytes: Lipolysis: Monounsaturated fatty acids: *n*-9 Fatty acids

Much interest has been shown over recent years in the biological value of monounsaturated fatty acids (MUFA), as they are the major component of olive oil (OL), which is supposedly responsible for some of the beneficial effects on health of the Mediterranean diet (Nestle, 1995; Serra Majem *et al.* 1995). Oleic acid (18:1*n*-9) is the major fatty acid in tissues such as muscle or adipose, though the proportion of 18:1*n*-9 can vary greatly from tissue to tissue (Soriguer *et al.* 2000).

Results of studies of the influence of dietary fatty acids on the lipolysis of adipocytes are contradictory. Most studies suggest that diets rich in polyunsaturated fatty acids (PUFA) promote greater lipolytic activity than diets rich in saturated fatty acids (SFA) (Su & Jones, 1993; Raclot & Oudart, 1999, 2000). However other studies have either found no difference between the effect of saturated and unsaturated fats on lipolysis (Mersman *et al.* 1992) or differences just between different saturated fats (Portillo *et al.* 1999) or even, under certain circumstances, a lower lipolytic activity of the unsaturated fats (Mersman *et al.* 1992). Recent studies, however, have shown that the molecular structure of fatty acids influences their release from the adipocyte, both *in vivo* and *in vitro*, with the mobilisation of the PUFA being greater than the MUFA and the SFA (Conner *et al.* 1996; Raclot & Oudart, 1999, 2000). A selective effect of fatty acids on the gene expression of the adipocytes has also been shown (Raclot & Oudart, 1999).

The specific effect of dietary MUFA on the lipolytic activity of the adipocyte has rarely been studied. In the essential fatty acid deficiency syndrome (EFAD) there is a characteristic increase in the MUFA in the tissues (Bjerve, 1989). Some studies in animal models of EFAD show both the baseline and the stimulated lipolytic activity of the adipocyte to be increased *in vitro* (Bizzi *et al.* 1967; Christ & Nugteren, 1970) and *in vivo* (Bergström & Carlson, 1965; DePury & Collins, 1965). These results do not appear to agree with the increase in lipolytic activity found in animals fed with a diet rich in essential long-chain fatty acids (Su & Jones, 1993; Raclot & Oudart, 1999, 2000).

In order to evaluate the role of *n*-9 fatty acids in the lipolytic activity of adipocytes *in vitro* the lipolytic activity of adipocytes was studied in two situations in which a cellular enrichment of monoenoic fatty acids was produced. One situation was based on a diet rich in oleic acid, and the other was due to an increase in monoenoic fatty acids resulting from the feeding of a deficit of essential fatty acids.

Materials and methods

Study design

The study was undertaken with forty-five male Sprague–Dawley rats (Griffa, S.A, Barcelona, Spain) divided into three groups of fifteen rats each, according to diet.

Abbreviations: EFAD, essential fatty acid deficiency syndrome; MUFA, monounsaturated fatty acids; OL, olive oil; PGE-2, prostaglandin E₂; PGI-2, prostacyclin; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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The animals were separated from their mothers at 3 weeks of age (one rat per cage to control food intake and faeces). During the whole study period these rats had free access to water and food. From weeks 3 to 6 the animals received a standard laboratory diet. From weeks 7 to 13 they were fed one of three equally isoenergetic diets. These diets differed only in their composition of fatty acids: in the first diet the fat came solely from palmitic acid (EFAD group); in the second it was a mixture of soyabean oil + palmitic acid; in the third it was OL.

The weight of the rats at the start of the study was 58.06 (SD 2.67) g, there being no *post hoc* differences between the groups.

The protocol was approved by the Ethics and Clinical Investigation Committee of the Carlos Haya Hospital.

Composition of the diets

The three diets provided the same amount of energy, and the concentrations of fat, protein, and carbohydrates were all similar. The only difference was in the quality of the fat. The fat concentration in all the diets was 80 g/kg. The source was palmitic acid in one (EFAD group), a mixture of 18% soyabean oil enriched with 82% palmitic acid in another, a proportion previously shown to be sufficient to prevent a deficit of essential fatty acids (Dulloo & Girardier, 1990; Tinahones *et al.* 1999), and OL in the third group. The fatty acid composition of the three diets is shown in Table 1. The remaining components were identical (g/kg diet): casein, 263; starch + sucrose, 588; mineral mix, 37; vitamins mix, 10; cellulose, 18; choline, 0.9; methionine, 3. The mineral mix supplied the following (g/kg diet): CaHPO₄, 18.4; NaCl, 2.7; potassium citrate, 8.1; K₂SO₄, 1.9; MgO, 0.9; MnCO₃, 0.13; ferric citrate, 0.22; ZnCO₃, 0.011; KI, 0.0004; Na₂SeO₃, 0.0004; CrKSO₄, 0.02. The vitamin mix supplied the following (mg/kg diet): thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine, 0.7; nicotinic acid, 3; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; vitamin B₁₂, 0.001; vitamins A + D, 1; vitamin E, 10; menadione, 0.005.

The materials for the preparation of the diets were obtained from Laboratorios Musal (Granada, Spain), Merck (Darmstadt, Germany) and Sigma Chemical Co.

Table 1. Fatty acid composition of the diets used (% fatty acids)

Fatty acid	Diet		
	Palmitic acid	Olive oil	Soyabean oil + palmitic acid
14:0	ND	ND	T
16:0	100	9.7	83.8
16:1 <i>n</i> -7	ND	0.9	T
18:0	ND	3.0	0.6
18:1 <i>n</i> -9	ND	78.8	4.5
18:2 <i>n</i> -6	ND	5.2	9.7
18:3 <i>n</i> -3	ND	0.6	1.2
20:0	ND	0.3	0.1
20:1 <i>n</i> -9	ND	0.3	0.1
20:5 <i>n</i> -3	ND	0.1	T
22:4 <i>n</i> -6	ND	0.1	T

ND, not detectable by GC; T, traces.

(St Louis, MO, USA). The energy content of the diets was 1724 kJ (412 kcal)/100 g, with the fats accounting for 17.5% of the total energy content.

Control of food intake and faeces

The food was weighed and renewed daily. The faeces were collected and weighed weekly and then frozen at -80°C for later study.

Energy balance

The energy contained in duplicate samples of the lyophilised carcass and faeces was measured by calorimetry (Parr Instrument Company, 1989, IL, USA) (Jühr & Franke, 1992). Energy balance was calculated for each animal individually as the difference between the total amount of energy consumed and the total amount of energy lost through the faeces.

Procedures

At the end of the study the animals were killed with CO₂ and carefully dissected immediately afterwards. Blood was obtained by intracardiac puncture and plasma leptin was measured by radioimmunoassay (Mediagnost, Reutlingen, Germany). The epididymal and omental adipose tissues were separated. The samples were weighed, and a part of each was then set aside for the measurement of the adipocyte volume and the lipolysis study. The rest was frozen at -80°C for further analysis. The remainder of each animal's body was also frozen at -80°C.

Separation of the adipocytes and calculation of their size and number

The separation of the adipocytes from the epididymal and omental tissues was made according to Robdell's method (Robdell, 1964). After extension on a silicon slide the diameter of 100 adipocytes was read and the mean and standard deviation calculated (DiGirolamo *et al.* 1971). The adipocyte volume was calculated assuming that the adipocytes were a sphere of fat, using Goldrick's equation (Goldrick, 1967). The total number of adipocytes in the different fat compartments was calculated from the total fat measured by gravimetry and the adipocyte volume, taking the density of fat to be 0.92 g/ml.

Fat concentration in the tissues and composition of fatty acids in the adipose tissue

The concentration of fat in each tissue sample was determined by gravimetry after extraction of the fat with chloroform-methanol (2:1, v/v) and butylated hydroxytoluene at 0.025% (p/v) (Folch *et al.* 1957).

Fatty acid methyl esters were formed by heating the extracted fat for 30 min with H₂SO₄ (0.61 M) in anhydrous methanol. After extraction with hexane, fatty acid methyl esters were analysed in a Hewlett-Packard chromatograph, equipped with a flame ionisation detector and using a BPX75 fused-silica capillary column (25 m × 0.32 mm,

0.25 μm film) (SGE, Villebon, France). The initial column temperature was 140°C, which was held for 1 min, and the injection and detector temperatures were 260 and 280°C respectively. The carrier gas was N_2 . Individual fatty acid methyl esters were identified by the comparison of their retention times with standards.

Measurement of lipolysis

The *in vitro* lipolytic activity induced by adrenaline, in both epididymal and omental adipose cells, was studied by stimulating lipolysis in cell cultures of 100 000 cells/ml and increasing concentrations of adrenaline (0–5000 nM), and by measurement of the concentration of glycerol in the culture medium. Briefly, after digestion of the adipose tissue with collagenase and separation of the cells, 1 ml cell suspension (100 000 cells/ml in Krebs–Henseleit medium with 5 mM-glucose) was placed in each culture plate well. After adding the corresponding concentration of adrenaline to each well, the plates were covered and incubated in a thermostatic bath at 37°C with gentle shaking for 2 h. After this incubation, the content of each well was transferred to a tube containing 200 μl silicone, and centrifuged for 30 s in a microcentrifuge. Finally, the infranatant fraction was recovered with a Pasteur pipette and frozen at -80°C until its use in the measurement of glycerol ($\mu\text{mol/l}$) by the GPO-Trinder method (Sigma Diagnostics, Inc., St Louis, MO, USA). The anti-lipolytic effect of the insulin was determined by fixing the concentration of adrenaline at 500 nM and using increasing concentrations of insulin (0–8000 pM), measuring the glycerol released in the medium (Vaughan & Steimberg, 1963; Fossati & Prencipe, 1982).

Statistical study

The data are presented as mean values and standard deviations. The means of the groups were compared by ANOVA. The tendency between variables was measured by Pearson's correlation coefficient (r). In all cases the level of rejection of a null hypothesis was $\alpha = 0.05$ for two tails.

Results

Somatometry

The final weight of the animals was greater in the OL and the EFAD groups, but the increase in weight was only greater in the OL group (Table 2). The total energy balance was significantly greater in the OL diet group (Table 2). The mean adipocyte volume of the epididymal tissue was approximately twice that of the omental tissue, with those animals fed OL having the highest adipocyte volume, both epididymal and omental (Table 2). There was, however, a close correlation between the mean size of the adipocytes in the epididymal and omental adipose tissues in the three groups (r 0.80, 0.90, 0.97 respectively; $P < 0.0001$).

Plasma leptin

The animals in the EFAD and OL groups had higher plasma leptin levels than those of the soyabean oil + palmitic-acid group. These differences were statistically significant after adjusting the differences for the adipocyte volume of the epididymal and omental adipose tissues, and for the final weight (Table 2). Though the plasma leptin values correlated either weakly or not at all with the final weight, there was a correlation in each of the three groups with the size of the epididymal adipocytes and especially with the size of the omental adipocytes (Table 3).

Fatty acid composition of adipose tissue

In both epididymal and omental tissues the greatest concentrations of MUFA occurred in the animals from the EFAD and OL groups. The highest concentration of PUFA was seen in the soyabean oil + palmitic-acid group (Table 4).

Baseline lipolysis

The baseline lipolysis was greater in the EFAD and OL animals than the soyabean oil + palmitic-acid animals (Table 5).

Table 2. Body weight, weight increase, energy balance (energy ingested minus energy in faeces during the experimental period), adipocyte volume, and plasma leptin of the study animals*
(Mean values and standard deviations)

Diet... Variable	Palmitic acid		Olive oil		Soyabean oil + palmitic acid		<i>P</i>
	Mean	SD	Mean	SD	Mean	SD	
Final weight (g)	388.2 ^b	36.8	410.3 ^a	18.7	355.3 ^b	28.9	0.001
Increase in weight (%)	132.8 ^b	23.7	149.0 ^a	6.6	136.4 ^b	18.1	0.04†
Energy balance (kJ)	10 669 ^b	697	15 316 ^a	2322	10 080 ^b	1119	0.0001†
Epididymal adipocytes volume (mm ³)	0.10 ^b	0.05	0.15 ^a	0.09	0.06 ^c	0.11	0.001
Omental adipocyte volume (mm ³)	0.05 ^b	0.03	0.08 ^a	0.02	0.04 ^b	0.01	0.001
Plasma leptin (ng/ml)	4.3 ^a	2.4	5.1 ^a	2.0	2.6 ^b	0.6	0.004†

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and p. 1016.

† Adjusted for the final weight of the rat (ANOVA).

Table 3. Linear correlation coefficients (Pearson's *r*) between plasma leptin and final weight and the fat cell size of the epididymal and omental adipose tissue†

Plasma leptin v. ...	Final weight	Epididymal adipocyte volume	Omental adipocyte volume
Palmitic-acid diet	0.77***	0.65**	0.62**
Olive-oil diet	0.04	0.50*	0.51*
Soyabean oil + palmitic-acid diet	0.32	0.76**	0.65**

P*<0.05, *P*<0.01, ****P*<0.001.

†For details of diets and procedures, see Table 1 and p. 1016.

Adrenaline-stimulated lipolysis

In the two tissues studied, the adrenaline-stimulated lipolysis was significantly greater for all the concentrations in the EFAD and OL animals (Fig. 1).

Inhibition of lipolysis by insulin

The inhibition of lipolysis by insulin was also lower for all the insulin concentrations in the EFAD and OL animals, in both the epididymal and omental tissues (Fig. 2).

Correlations

There was a positive correlation between the MUFA concentrations in the epididymal and omental tissues, and a

Table 4. Concentration of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in epididymal and omental adipose tissues† (Mean values and standard deviations)

Diet* ...	Palmitic acid		Olive oil		Soyabean oil + palmitic acid	
	Mean	SD	Mean	SD	Mean	SD
Epididymal adipose tissue						
SFA (%)	39.1 ^a	1.6	27.3 ^b	2.2	38.8 ^{a,b}	1.0
MUFA (%)	50.0 ^b	3.0	59.9 ^a	3.4	44.9 ^c	1.8
PUFA (%)	7.5 ^b	2.8	8.2 ^{ab}	1.2	12.0 ^a	1.6
Omental adipose tissue						
SFA (%)	41.0 ^a	1.4	27.3 ^b	1.9	40.4 ^a	1.0
MUFA (%)	51.2 ^b	2.0	61.9 ^a	2.7	45.2 ^c	2.1
PUFA (%)	3.6 ^c	2.0	6.7 ^b	1.9	9.9 ^a	1.3

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different (*P*<0.05).

*In all cases, the inter-diet differences (ANOVA) were highly significant (*P*<0.0001).

†For details of diets and procedures, see Table 1 and p. 1016.

negative correlation of the PUFA with the area under the curve of the glycerol response with increasing amounts of adrenaline (Table 6). These correlations remained after adjusting the correlation coefficients for the mean adipocyte volume (Table 6).

Discussion

An *in vitro* study was undertaken of the role of *n*-9 MUFA in adipocyte lipolysis using an experimental model in which the levels of *n*-9 MUFA were raised in adipose tissue by two different ways. One way was by increasing the *n*-9 MUFA in the diet; the second way was by an endogenous increase in *n*-9 MUFA in animals in which a deficiency of essential fatty acids was induced.

The increase in the lipolytic response in the rats fed with OL and in the EFAD rats, as well as the close correlation of *n*-9 MUFA with the tissue concentration, suggests that enrichment of *n*-9 MUFA in the adipocytes increases both their baseline and adrenaline-stimulated lipolytic activity.

The essential character of some fatty acids and the clinical importance of their deficiency have long been known (Hansen *et al.* 1963; Holman, 1977; Holman *et al.* 1982; Bjerve, 1991). There were studies during the 1970s of lipolytic activity in rats submitted to a deficiency of essential fatty acids (Bergström & Carlson, 1965; DePury & Collins, 1965; Bizzi *et al.* 1967; Christ & Nugteren, 1970). However, the authors of the present study have been unable to find any references in Medline or Embase to later studies confirming these results and which specifically study lipolytic activity of adipocytes in rats with EFAD. In the present study, the baseline lipolysis of the adipose cell and lipolysis induced by adrenaline were greater in those animals exposed to a deficiency of fatty acids. Although insulin is known to be able to inhibit the lipolytic response

Table 5. Baseline lipolysis: glycerol values without addition of adrenaline to the medium (Mean values and standard deviations)

Diet ...	Palmitic acid		Olive oil		Soyabean oil + palmitic acid		<i>P</i>
	Mean	SD	Mean	SD	Mean	SD	
Baseline glycerol (epididymal adipose tissue) (μmol/l)	107.1 ^a	32.7	78.9 ^b	12.4	37.2 ^c	7.3	0.001†
Baseline glycerol (omental adipose tissue) (μmol/l)	118.9 ^a	38.1	70.8 ^b	17.1	47.8 ^c	13.2	0.001†

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different (*P*<0.05).

*For details of diets and procedures, see Table 1 and p. 1016.

†Adjusted for adipocyte volume.

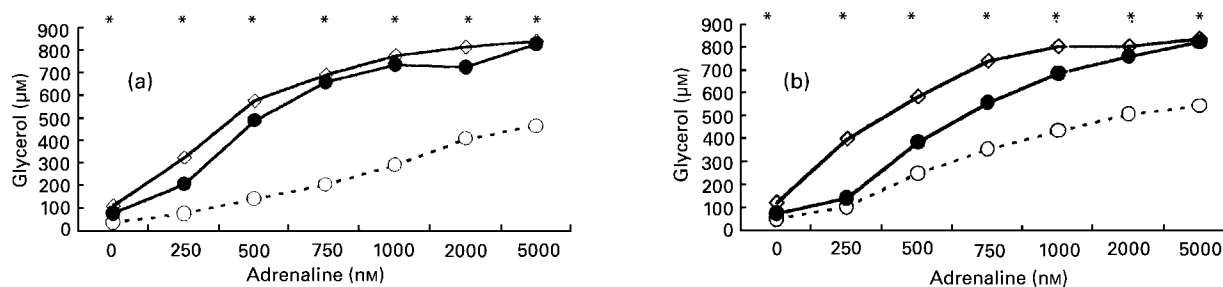


Fig. 1. Lipolytic activity of the adipocytes measured by the production of glycerol with increasing amount of adrenaline in (a) adipocytes of the epididymal tissue, and (b) adipocytes of the omental tissue. (—◇—), Palmitic-acid diet; (—○—), soyabean oil + palmitic-acid diet; (—●—), olive-oil diet. **P* < 0.0001.

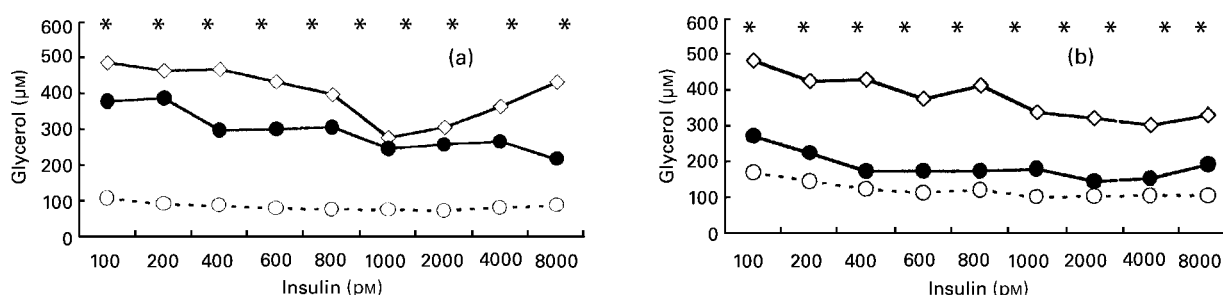


Fig. 2. Lipolysis inhibition by different insulin concentrations (500 µM-adrenaline) in (a) adipocytes of the epididymal tissue, and (b) adipocytes of the omental tissue. (—◇—), Palmitic-acid diet; (—○—), soyabean oil + palmitic-acid diet; (—●—), olive-oil diet. **P* < 0.0001.

induced by adrenaline (McMurchie *et al.* 1987), this anti-lipolytic capacity was lower in the EFAD animals.

These results appear to contradict those that demonstrate that the lipolytic activity of the adipocytes is greater in animals fed with a diet rich in PUFA (Awad & Chattopadhyay, 1986; McMurchie *et al.* 1987; Parrish *et al.* 1991; Hill *et al.* 1993; Su & Jones, 1993). This is because in the absence of PUFA the opposite would be expected, i.e. a reduction in lipolytic activity. However, these studies were undertaken by increasing the intake of PUFA and comparing this with a diet rich in SFA with a sufficient proportion of *n*-3 or *n*-6 fatty acids so as not to produce the EFAD syndrome, and therefore an increase in *n*-9 fatty acids. In EFAD animals the characteristic tissue pattern is an increase in *n*-7 and *n*-9 fatty acids coupled with a decrease in *n*-6 fatty acids (Holman, 1977). As was to be expected, in the present study the EFAD rats had a greater concentration of MUFA and a lower concentration of PUFA, in both the epididymal and the omental adipose tissues. Of note was the close correlation between

the lipolytic response (expressed as the area under the curve) and the concentration of MUFA in the adipose tissue. This relationship persisted even after adjustment with the mean volume of the epididymal and omental adipocytes, because lipolysis, at least baseline lipolysis, is known to be able to be conditioned by the size of the adipocytes (Zinder & Hapiro, 1971; Smith *et al.* 1979).

As expected, in the animals fed with OL there was also an increase in *n*-9 fatty acids in the adipose tissues (Sorrigue *et al.* 2000). In this group of animals the lipolytic response at baseline and after adrenaline was nearer that of the EFAD animals than that of those fed with a mixture of SFA and PUFA (soyabean oil + palmitic acid), as also occurred with the lower anti-lipolytic action of insulin. Reports of the effect that the type of dietary fat has on lipolytic capacity in plasma and adipose tissue differ. Most suggest that diets rich in PUFA promote greater lipolytic activity than saturated fats (Su & Jones, 1993; Eynard *et al.* 1998). On the other hand, some have found no difference on lipolysis between SFA and PUFA (Mersman *et al.* 1992), or just between different SFA (Portillo *et al.* 1999) or even, under particular circumstances, a lower lipolytic activity of the PUFA (Mersman *et al.* 1992). However, most studies were not oriented to evaluating the role of the *n*-9 fatty acids in lipolytic activity, but rather the *n*-6 or *n*-3 fatty acids.

The use of two different induction models to increase the levels of *n*-9 fatty acids in the tissues helped to exclude other possible confounding factors. These confounding factors could be the decrease in *n*-6 fatty acids, which was much less in the OL rats than in the EFAD rats, or the clinical state of the animals (in the EFAD group), or possible unidentified effects of some of the dietary components,

Table 6. Linear correlation coefficients (Pearson's *r*) between the area under the curve of lipolysis stimulated by adrenaline and the sum of the saturated fatty acids (SFA), the monounsaturated fatty acids (MUFA) and the polyunsaturated fatty acids (PUFA) in adipose tissues adjusted for adipocyte volume†

Area under the curve of lipolysis v. ...	SFA	MUFA	PUFA
Epididymal adipose tissue	-0.29	0.43*	-0.47*
Omental adipose tissue	-0.28	0.48*	-0.69***

P* < 0.05, **P* < 0.001.

† For details of procedures, see p. 1017.

which cannot be controlled for either in study design or in statistical analysis. The use of the two induction models was also to minimise other confounding variables such as the weight of the animals, the energy balance, or the adipocyte volume, which were on the other hand taken into account in the hypothesis contrast.

The possibility that enrichment of *n*-9 fatty acids may be influential in a greater lipolytic response cannot be discarded. The mechanisms by which enrichment with *n*-9 fatty acids in the tissues induce greater lipolytic activity should be investigated. Rodríguez *et al.* (2002*b*) have shown that OL induces an up regulating effect on uncoupling protein mRNA that is probably not mediated by systemic metabolic changes. Furthermore the role of leptin in the regulation of lipolysis has recently been described (Wang *et al.* 1999). In cultures of adipocytes it has been seen that, whereas adrenaline increases both glycerol and fatty acids, leptin induces a release of glycerol but not of fatty acids. This suggests a new form of leptin-mediated lipolysis. The possibility cannot be ruled out that the *n*-9 fatty acids induce a modification of leptin metabolism and that, either alone or interacting with lipolytic hormones, they can modify lipolytic activity (Rodríguez *et al.* 2002*a*). Greater accumulations of adipose tissue can increase the levels of circulating leptin, which in turn would increase adipose tissue lipolytic activity (Martínez *et al.* 2000). Indeed, in the present study, the plasma leptin was higher in those animals with an increase in *n*-9 fatty acids in the adipose tissue, even after adjusting the levels of leptin for adipocyte volume, both epididymal and omental, there being a moderate statistical correlation (r 0.30; $P=0.01$) between leptin levels and MUFA in the adipose tissue. Obici *et al.* (2002) have shown that the central administration of oleic acid produces an increase in insulin sensitivity at the same time as it reduces plasma insulin levels. The authors suggest that the leptin–insulin balance might determine the rate of lipolysis *in vivo*.

Recent studies have shown that the treatment of pre-adipocyte cells with fatty acids, especially PUFA, induces the expression of several genes encoding proteins related to fatty acid metabolism. The mechanism of gene expression in adipocytes appears to differ, at least partly, from that in the liver (Raclot & Oudart, 1999). Although these studies were undertaken examining the effect of PUFA on the expression of genes related to fatty acid metabolism, especially in the liver, it is possible that the different lipolytic activity of adipocytes enriched with *n*-9 fatty acids can be explained by the specificity of the different fatty acids in gene expression.

Hydrolysis of fatty acids from adipose tissue triacylglycerols has been shown, *in vivo* (Conner *et al.* 1996) and *in vitro* (Raclot & Oudart, 1999), to be specific for each fatty acid, and the mobilisation generally correlates positively with unsaturation and negatively with the length of the chain. Furthermore, during lipolysis, there is also a specific recapture and re-esterification of the fatty acids by adipocytes (Raclot & Oudart, 1999).

In the present study the anti-lipolytic capacity of insulin was decreased in the adipocytes of those animals with greater levels of *n*-9 fatty acids in adipose tissue. The

anti-lipolytic effect of insulin is mediated by its capacity to act on cyclic phosphodiesterase, and via this to regulate levels of cAMP. Zhang *et al.* (1993) showed that adrenaline induces the release by the adipocytes of prostaglandin E₂ (PGE-2) and prostacyclin (PGI-2), in parallel with the production of glycerol, and that this effect is potentiated by the presence of stromal cells in the medium, especially in the presence of high concentrations of essential fatty acids. In the present study the adipose cells were washed several times before being cultured, but it cannot be ruled out that there were no stromal cells, which might have acted in co-operation with the adipocytes. It is possible that enrichment with MUFA produces changes in the amount or functionality of stromal cells and that part of the observed effect was due to the stromal tissue and not wholly to the adipose tissue. Previous studies by our group (Tinahones *et al.* 1999) and others (Hjelte *et al.* 1990) have shown that in rats with EFAD the secretion of insulin by the islets of Langerhans is abnormal, both *in vitro* and *in vivo*. Herrera *et al.* (2000) showed that the lipolytic response to β -3 agonist receptors by adipocytes is worsened in rats with chronic hyperinsulinaemia and that this response can be modified by changing the dietary composition of the phospholipids of the adipocytes. A disorder in insulin metabolism *in vivo* in rats with MUFA-enriched tissues could explain the lesser *in vitro* lipolytic effect, for example via a mechanism of down regulation of the number or of the affinity of the insulin receptors for the adipocyte.

PGE-2 and PGI-2 are produced by the adipocytes, with PGE-2 exercising a marked anti-lipolytic effect *in vitro* and *in vivo*, and PGI-2 a marked lipolytic effect in the presence of stromal cells (Chatzipanteli *et al.* 1992). Both substances are produced from arachidonic acid, so that it is not surprising that in EFAD animals the release of PGE-2 is diminished (Christ & Nugteren, 1970). An imbalance in the production of PGE-2 and PGI-2 by the adipose cells from animals with a high content in *n*-9 fatty acids could explain their greater lipolysis. Also, the production of both PGE-2 and PGI-2 by adipose tissue is inhibited by insulin (Axelrod *et al.* 1986).

In conclusion, the results of the present study confirm that in situations in which there is an increase of *n*-9 fatty acids in adipocytes the lipolytic activity of the adipocytes is increased and the anti-lipolytic capacity of the insulin decreased. This increase in lipolytic activity in EFAD animals could, teleologically, be a defence mechanism against a deficit of essential fatty acids, in an attempt to release most of their essential fatty acids from their depots. Although extreme deficiency is rare nowadays in human populations, partial deficiencies of essential fatty acids have been reported in numerous clinical situations (Holman *et al.* 1981, 1995; Clandinin *et al.* 1983; Strandvik *et al.* 1988; Bjerve, 1989; Nicolas *et al.* 1990; Makrides *et al.* 1995; Tinahones *et al.* 1999). The recognition of this greater lipolytic effect of the adipose tissue in situations associated with EFAD and its relationship with the increase in *n*-9 fatty acids may be useful to better understand the physiopathology of certain clinical entities, as well as to design better and more precise therapeutic regimens.

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