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Dietary oleic acid and adipocyte lipolytic activity in culture☆

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Abstract

Although various studies have noted fatty-acid-mediated regulation of adipocyte lipolysis, determining the isolated effect of a single fatty acid is more difficult. We examined the influence of dietary oleic acid on adipose cell lipolytic activity and the tissue fat content independently of the variation in other dietary fatty acids. We fed 48 rats with six diets designed so that the oleic acid content was not correlated with the content of any other fatty acid and studied the lipolytic activity and fatty acid content of the tissues. There were no differences in the weight of the animals after the diet. The muscle fat content and the epinephrine-stimulated lipolytic activity varied significantly according to the dietary levels of oleic acid and the tissues, showing a dose-dependent behavior of the dietary oleic acid concentration. The results of this study show that diets rich in oleic acid have a beneficial effect on the regulation of lipid metabolism and body weight homeostasis.

Keywords: Lipolysis; Oleic acid; Muscle fat

1. Introduction

Fat is stored mainly in adipose tissue, although fat depots also exist in the liver and muscle. The existence of fat depots in the muscle has been directly associated with a greater risk for obesity, insulin resistance and Type 2 diabetes mellitus [1,2]. The regulation of adipose tissue and muscle fat content is therefore paramount for body weight homeostasis.

Adipocyte lipolysis is the process by which triglycerides in the adipose tissue are hydrolyzed, resulting in 3 mol of free fatty acids and one of glycerol for each mole of fully hydrolyzed triglyceride. The basic enzyme involved in this process is hormone-sensitive lipase (HSL) [3]. This process is regulated hormonally by insulin and catecholamines. The catecholamines activate the β-adrenergic receptors of the adipocyte, thereby initiating the lipolytic cascade, whereas insulin binds to its receptors and inhibits lipolysis [3]. Genetic regulation exists via the PPARα, whose main catabolic activity favors the phenomena of fatty acid β-oxidation and triglyceride hydrolysis mediated by induction of lipoprotein lipase [4,5]. This induction of lipolysis by PPARα is also mediated by the action of leptin [6], which is another genetic factor regulating the lipolytic process.

Adipocyte lipolytic activity is also influenced by nonhormone factors. A previous study by our group showed that dietary oleic acid is associated with adipocyte lipolytic activity in vitro [7], and later studies detected regulation in adipocyte lipolysis mediated by fatty acids [4], with a reduction in the accumulation of fat in the visceral adipose tissue in obese rats [8] and epididymal adipose tissue in mice [9]. However, in vivo, the isolated effect of the increase in one dietary fatty acid is difficult to separate from the reduction of another in a similar proportion to that being studied. Accordingly, we examined the influence of dietary oleic acid on adipose cell lipolytic activity and on the tissue

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Table 1
Fatty acid composition of the diets

Fatty acid	Fatty acid composition of the diets (%)					
	Coconut	Coconut–sunflower	Sunflower	Coconut–olive	Olive–sunflower	Olive
Caprilic	1.14	1.41	–	0.80	–	–
Capric	4.52	2.57	–	0.05	–	–
Lauric	46.30	22.92	–	22.74	–	–
Myristic	19.47	9.54	–	9.57	–	–
Palmitic	11.68	9.00	8.06	11.31	9.80	9.70
Stearic	3.90	4.03	4.93	3.76	4.14	3.00
Oleic	9.54	18.24	30.49	45.11	54.88	79.00
Linoleic	3.46	32.29	56.52	6.66	31.18	5.20

fat content independently of the variation of other dietary fatty acids.

2. Materials and methods

The study was undertaken with 48 male Sprague–Dawley rats with an initial weight of 100–125 g (Charles River, Barcelona). The animals were caged individually at a constant temperature and 12-h light/dark cycles, with free access to food and water. The animals were randomly assigned to six different diets for 4 weeks (7–8 animals per diet). The diets were designed so that the content of oleic acid did not correlate with the content of any other fatty acid. The diets were based on the AIN-93M diet for rodents [10] with 8% total fat and an identical composition except for the oil: coconut oil, olive oil, sunflower oil or a 50% mixture (coconut + olive, coconut + sunflower, or olive + sunflower). Table 1 shows the fatty acid composition of each diet.

The use of these animals in the experimental protocols of this study was approved by the Ethics and Clinical Research Committee of the Carlos Haya Hospital.

The food consumption was calculated as the difference between that supplied over the whole study period and the amount remaining at the end of the study. No differences existed in the consumption of food between the diets (data not shown). The rats were weighed at the beginning and the end of the study period.

The animals were sacrificed by anesthesia with CO_2. After dissection, the amounts of epididymal and omental adipose tissues were weighed, and a sample was taken from each of these tissues plus some abdominal muscle tissue to measure the fatty acid composition and the percentage of fat in each [11] and for gene expression studies. Fatty acid composition of the tissues was measured by total lipid extraction [11], thin layer chromatography

Table 2
Differences in the study variables depending on the diet

	Percentage of dietary oleic acid						*P*
	9.5%	18.2%	30.5%	45.1%	54.9%	79.0%	
Rat final weight (g)	318±20	313±29	312±28	298±30	322±27	320±23	ns
Epididymal adipose tissue							
Volume ($\mu m^3 \times 10^4$)	19±3	20±3	21±5	21±4	21±2	23±3	ns
Number ($\times 10^{10}$)	2.2±0.6	1.9±0.5	1.6±0.3	1.8±0.4	2.2±0.5	1.7±0.2	ns
% Fat	92.2±6.0	92.0±7.7	92.1±6.1	91.0±5.5	96.1±2.5	89.7±6.6	ns
% Saturated	56.2 ± 1.5^{a}	41.2 ± 2.7^{b}	25.5 ± 1.8^{d}	39.1 ± 1.6^{c}	24.6 ± 0.9^{d}	26.6 ± 3.6^{d}	<.001
% Oleic	29.0 ± 1.6^{a}	26.6 ± 1.4^{a}	28.9 ± 0.7^{a}	48.8 ± 2.2^{b}	47.9 ± 1.2^{b}	63.1 ± 5.2^{c}	<.001
% Polyunsaturated	3.6 ± 0.7^{d}	24.5 ± 1.6^{b}	40.7 ± 2.7^{a}	4.3 ± 0.7^{d}	22.6 ± 1.2^{c}	4.3 ± 0.7^{d}	<.001
Leptin	0.5±0.3	2.1±2.1	0.4±0.2	0.9±0.5	1.2±1.2	1.6±1.0	.06
HSL	0.03±0.01	0.05±0.06	0.04±0.03	0.06±0.03	0.06±0.05	0.04±0.02	.7
PPARα	0.2±0.1	0.5±0.5	0.2±0.1	0.3±0.2	0.3±0.3	0.2±0.2	.4
Omental adipose tissue							
Volume ($\mu m^3 \times 10^4$)	1.0±0.3	1.1±0.2	1.3±0.4	1.1±0.2	1.1±0.2	1.2±0.4	ns
Number ($\times 10^{10}$)	2.6±0.8	2.3±0.8	2.4±0.2	2.2±0.7	2.1±0.5	2.1±0.3	ns
% Fat	87.5±7.4	89.8±5.98	88.3±6.4	86.0±4.6	89.0±6.7	85.9±6.6	ns
% Saturated	56.7 ± 1.8^{a}	42.1 ± 1.7^{b}	26.6 ± 2.9^{c}	40.5 ± 1.7^{b}	26.7 ± 3.3^{c}	25.6 ± 1.8^{c}	<.001
% Oleic	29.9 ± 1.8^{b}	$28.0\pm0.5^{a,b}$	29.7 ± 0.7^{a}	49.3 ± 1.6^{c}	49.2 ± 1.4^{c}	65.8 ± 3.1^{d}	<.001
% Polyunsaturated	3.5 ± 0.8^{c}	23.7 ± 1.8^{b}	39.5 ± 3.8^{a}	4.1 ± 1.1^{c}	22.3 ± 2.4^{b}	4.3 ± 0.5^{c}	<.001
Leptin	0.3±0.1	0.5±0.5	0.1±0.1	0.2±0.1	0.3±0.31	0.4±0.2	.2
HSL	0.02±0.01	0.05±0.03	0.03±0.01	0.03±0.02	0.03±0.01	0.03±0.02	.1
PPARα	$0.2\pm0.2^{a,b}$	0.3 ± 0.1^{b}	0.1 ± 0.1^{a}	$0.28\pm0.08^{a,b}$	0.14 ± 0.07^{a}	$0.23\pm0.09^{a,b}$	.04
Muscle tissue							
% Fat	10.9 ± 4.0^{b}	4.7 ± 2.9^{a}	4.5 ± 2.2^{a}	4.5 ± 2.3^{a}	3.9 ± 1.6^{a}	3.4 ± 1.6^{a}	<.001
% Saturated	52.5 ± 2.3^{a}	41.7 ± 2.3^{b}	29.8 ± 3.7^{c}	38.9 ± 1.9^{b}	29.3 ± 2.1^{c}	28.1 ± 5.6^{c}	<.001
% Oleic	$31.5\pm2.7^{a,b}$	27.2 ± 2.9^{a}	33.9 ± 2.3^{b}	47.8 ± 2.6^{d}	42.4 ± 6.4^{c}	61.1 ± 6.9^{e}	<.001
% Polyunsaturated	4.0 ± 1.1^{c}	22.2 ± 7.3^{b}	31.8 ± 5.0^{a}	4.1 ± 1.5^{c}	21.7 ± 6.2^{b}	4.8 ± 2.7^{c}	<.001

% Fat, percentage of fat in the tissues; % Saturated, percentage of the total saturated fatty acids in the tissues; % Oleic, percentage of oleic acid in the tissue; % Polyunsaturated, percentage of the total polyunsaturated fatty acids.
Values are means±S.E.M. (n=48). Different letters represent different significant means.

(hexan/diethyl ether/acetic acid, 80:20:2) and gas chromatography in chromatograph HP 6890 (Hewlett- Packard, Palo Alto, CA) and column Sulpeco 24152 (0.25 μm, 30 m and 0.32 mm). The percentage of fat in each tissue was calculated as the ratio between the lipidic extract weight and the sample weight.

The adipocytes from the epididymal and omental tissues were isolated by enzyme digestion [12] in KRBHA buffer with collagenase; the cell volume was calculated using the Goldrick [13] equation, and the number of adipocytes was determined from the DiGirolamo equation [14]. The lipolytic activity of the adipocytes was studied in vitro, measured by the amount of glycerol spontaneously released to the medium during 2 h and at two concentrations of epinephrine (500 and 2000 nmol/L) to stimulate lipolysis. The lipolysis inhibition was studied at a fixed concentration of epinephrine (500 nmol/L) and at three concentrations of insulin (0.5, 3 and 6 nmol/L). The results were expressed as the concentration of glycerol measured by a commercial kit Randox (Lab, UK) in the culture medium.

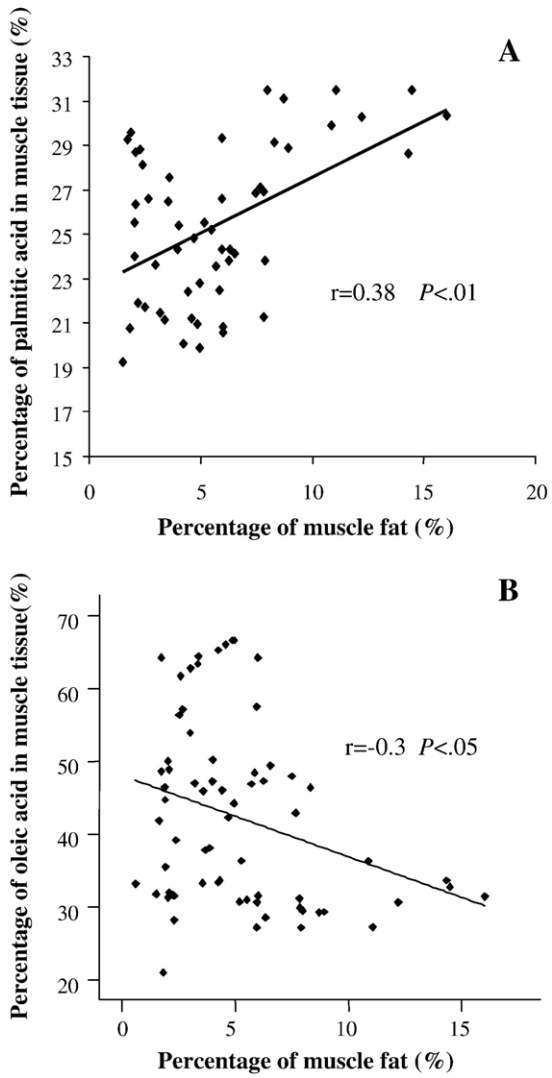

Fig. 1. Percentage of fatty acids in the muscle according to the percentage of muscle fat. Correlation calculated with Spearman test. (A) Percentage of palmitic acid. (B) Percentage of oleic acid.

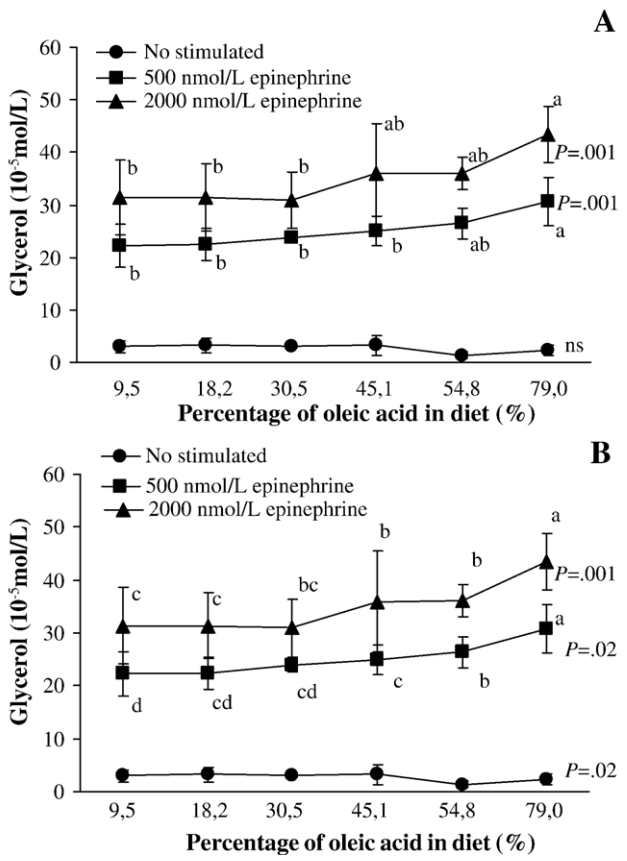

Fig. 2. Adipocyte lipolysis according to the percentage of dietary oleic acid. The values are presented as means±S.E.M. (n=48). Different letters represent significantly different means. (A) Lipolysis in epididymal adipose tissue. (B) Lipolysis in omental adipose tissue.

The gene expression levels relative to the β-actin expression of HSL, PPARα and leptin were studied in the intact epididymal and omental tissues by real-time PCR. The primers used were: s-TCTACAATGAGCTGCGTGTG, a-ACGCTCGGTCAGGATCTTC (β-actin), s-GCATGGATTTACGCACAATG, a-AGTTGGTTCTAGCCCCAGTG (HSL), s-TCACACAATGCAATCCGTTT, a-GGCCTTGACCTTGTTCATGT (PPARα) and s-CCTGTGGCTTTGGTCCTATCTG, a-AGGCAAGCTGGTGAGGATCTG (leptin).

The data are presented as the mean and standard deviation for each diet. Comparisons were made by one-way ANOVA or by the nonparametric Kruskal–Wallis test. Differences between means were compared by the Duncan post hoc test. Associations between variables were measured with the Pearson or Spearman correlation coefficient, according to their adjustment to normality. In all cases, the level of rejection for a null hypothesis was α=.05 for two tails. The dose-response studies were done with a trend test [15].

3. Results

Table 2 shows the mean values of the study variables according to the diet received. No significant differences were found in total weight, weight of the intra-abdominal adipose tissue, adipocyte volume, total number of adipocytes or percentage of adipose tissue fat. The content of muscle tissue fat, however, was significantly greater in the group fed with coconut oil, showing an inverse linear correlation with the proportion of dietary oleic acid (P for trend=.001). The fatty acid composition in the tissues varied significantly between diets, depending on their fatty acid composition (Table 2).

The muscle tissue content of oleic acid showed an inverse correlation with the proportion of fat in the tissue (r=−.30, P=.05) and a direct correlation with the content of lauric (r=.46 P=.01), myristic (r=.52 P=.01) and palmitic (r=.38 P=.01) fatty acids (Fig. 1). No significant differences were found in gene expression levels according to the diet (Table 2).

The epinephrine-induced lipolysis of the epididymal adipose tissue adipocytes was greater in animals fed with diets having a greater proportion of oleic acid (P<.001). The omental tissue adipocytes showed a similar association (P=.02) (Fig. 2). These increases in epinephrine-induced lipolytic activity in both tissues were dose-dependent on the dietary concentration of oleic acid (P for trend <.01 in all cases).

The adipocyte lipolytic activity in the epididymal and omental tissues showed a direct correlation with the proportion of oleic acid in the adipose tissues (Table 3) and an inverse association with the tissue content of saturated and polyunsaturated fatty acids. The gene expression levels did not correlate with the lipolytic activity in any of the tissues (Table 3).

4. Discussion

Fat is mainly stored in adipose tissue, although it is also accumulated in muscle tissue and the liver [16]. The inability to increase fat oxidation in intramyocellular fat depots may play a decisive role in the development of insulin resistance in the muscle, as well as in the pathogenesis of obesity [17–19].

This study showed an inverse, dose-dependent association between the dietary content of oleic acid and the percentage of muscle tissue fat. This association was corroborated by the presence of a significant inverse correlation between the tissue content of oleic acid and its fat content. Thus, a diet rich in oleic acid might prevent the development of insulin resistance induced by muscle tissue fat and the resulting development of obesity and Type 2 diabetes mellitus.

Data reported by Lee [20] showed that the accumulation of saturated fatty acids in muscle tissue induces insulin resistance. This study showed that the content of saturated lauric, myristic and palmitic fatty acids did indeed have a direct correlation with the percentage of muscle fat. This may be due to a greater accumulation of fat induced by the dietary saturated fatty acids or a reduction in lipolysis or oxidation of the muscle lipids.

Adipose cell lipolytic activity can be modulated by many variables, with those related with the diet being of great importance [21]. We have already shown in rats that enriching tissues with monounsaturated fatty acids, either from the diet or from the de novo synthesis of palmitic acid, is associated with the lipolytic activity of adipose cells cultured in vitro and that this lipolytic activity has a negative correlation with the percentage of polyunsaturated fatty acids in adipose tissue [7]. The results of the present study

Table 3
Correlations between the lipolysis of the in vitro cultured adipocytes at different concentrations of epinephrine, with the tissue fatty acid composition and the gene expression levels

	Fatty acids composition in the different adipose tissues								HSL	Leptin	PPARα
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1n9	C18:2n6	C20:4n6			
Epididymal adipose tissue											
BL (μmol/L)	0.39[a]	0.30	0.03	0.19	−0.11	−0.22	0.001	0.02	−0.02	−0.002	0.02
L500 (μmol/L)	−0.46[b]	−0.48[b]	−0.37[a]	−0.25	−0.17	0.61[b]	−0.14	−0.45[b]	0.07	0.10	−0.10
L2000 (μmol/L)	−0.25	−0.31	−0.26	−0.10	−0.21	0.59[b]	−0.31	−0.44[b]	0.05	0.13	−0.02
HSL	−0.03	0.04	0.25	0.04	0.24	0.14	−0.17	−0.01	–	–	–
Leptin	−0.15	−0.10	0.15	−0.05	−0.005	0.27	−0.14	−0.25	–	–	–
PPARα	0.04	0.10	0.20	0.01	0.22	−0.05	0.007	−0.01	–	–	–
Omental adipose tissue											
BL (μmol/L)	0.10	0.11	−0.001	0.14	−0.27	0.15	−0.18	−0.25	0.07	−0.29	0.06
L500 (μmol/L)	−0.44[b]	−0.51[b]	−0.28	−0.23	−0.08	0.73[b]	−0.29	−0.36[a]	0.21	0.24	0.13
L2000 (μmol/L)	−0.55[b]	−0.60[b]	−0.39[b]	−0.34[a]	−0.001	0.66[b]	−0.16	−0.40[b]	0.12	0.05	−0.15
HSL	0.02	0.01	−0.14	−0.06	0.03	−0.14	0.17	0.12	–	–	–
Leptin	0.03	0.13	0.30	0.28	−0.29	−0.01	−0.27	0.07	–	–	–
PPARα	0.23	0.27	0.21	0.21	−0.10	−0.20	−0.05	0.07	–	–	–

BL, baseline lipolysis; L500, lipolysis stimulated with 500 nmol/L of epinephrine; L2000, lipolysis stimulated with 2000 nmol/L of epinephrine. C12:0, lauric; C14:0, myristic; C16:0, palmitic; C16:N1, palmitoleic; C18:0, stearic; C18:1N9, oleic; C18:2N6, linoleic; C20:4N6, arachidonic.

[a] The correlation was significant at .05.

[b] The correlation was significant at .01.

support our earlier data [7], with the finding of strong direct correlations between the levels of lipolysis stimulated by different concentrations of epinephrine and the proportion of oleic acid in epididymal and omental tissues, and inverse relations of the saturated fatty acids and the content of polyunsaturated arachidonic acid. In addition, this study also showed a dose-dependent association between the oleic acid content of the different diets and the lipolytic activity of adipose cells cultured in vitro, in both epididymal and omental tissues, at all the concentrations of epinephrine used to stimulate the lipolysis. As the baseline lipolysis (with no epinephrine in the medium) was not influenced by the dietary oleic acid, this effect may occur in the adipocyte adrenergic receptors or at some point in the chain of events generating its activation.

In an attempt to explain the mechanism of action of oleic acid on lipolysis, we examined the expression levels of the PPARα, leptin and HSL genes in epididymal and omental adipose tissues. The expression levels of these genes did not vary according to the diet or correlate with the tissue levels of oleic acid or the increase in lipolytic activity.

A worsening in the lipolytic response in adipose tissue is associated with obesity [22], so lipolysis is a target for its control. However, excess lipolytic activity contributes to an increase in the circulating levels of free fatty acids, leading to the development of dyslipidemia characteristic of the metabolic syndrome [21,23]. Thus, the use of lipolysis activators has been proposed to combat these disorders; these activators act stimulating both the oxidation of fatty acids in skeletal muscle and energy expenditure [19].

The results of this study show that both the percentage of muscle fat and the levels of lipolysis stimulated by epinephrine vary greatly with the diet, having a dose-dependent behavior according to the dietary concentration of oleic acid and correlated with the tissue content of oleic acid. Therefore, diets rich in oleic acid may have a beneficial effect on the regulation of lipid metabolism and the homeostasis of body weight.

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