Serum adipokine profile and fatty acid composition of adipose tissues are affected by conjugated linoleic acid and saturated fat diets in obese Zucker rats

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Conjugated linoleic acid (CLA) has been reported as having body fat lowering properties and the ability to modulate the inflammatory system in several models. In the present study, the effects of CLA added to saturated fat diets, from vegetable and animal origins, on the serum adipokine profile of obese Zucker rats were assessed. In addition, the fatty acid composition of epididymal and retroperitoneal adipose tissues was determined and a principal component analysis (PCA) was used to assess possible relationships between fatty acids and serum metabolites. Atherogenic diets (2 % cholesterol) were formulated with palm oil and ovine fat and supplemented or not with 1 % of a mixture (1:1) of *cis*-9,*trans*-11 and *trans*-10,*cis*-12-CLA isomers. CLA-fed animals exhibited lower daily feed intake, final body and liver weights, and hepatic lipids content. Total and LDL-cholesterol levels were increased in CLA-supplemented groups. CLA also promoted higher adiponectin and lower plasminogen activator inhibitor-1 (PAI-1) serum concentrations. In contrast to palm oil diets, ovine fat increased insulin resistance and serum levels of leptin, TNF-α and IL-1β. Epididymal and retroperitoneal adipose tissues had similar deposition of individual fatty acids. The PCA analysis showed that the *trans*-10,*cis*-12-CLA isomer was highly associated with adiponectin and PAI-1 levels. Summing up, CLA added to vegetable saturated enriched diets, relative to those from animal origin, seems to improve the serum profile of adipokines and inflammatory markers in obese Zucker rats due to a more favourable fatty acid composition.

Conjugated linoleic acid: Saturated diets: Adipokines: Fatty acids: Obese Zucker rats

The metabolic disorders caused by overweight are one of the major factors contributing to the increase in health care costs. Among these disorders are atherosclerosis, hypertension, insulin resistance, hypertriglyceridaemia and hypercholesterolaemia[1]. Dysregulated endocrine function of adipose tissue, in particular of visceral compartment, leads to an increased release of hormones and pro/anti-inflammatory molecules[1]. These factors secreted by adipose tissue, although not exclusively by adipose cells[2], are called adipokines. They include adiponectin, leptin, TNF-α, IL-1β and IL-6, C-reactive protein (CRP), monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 (PAI-1), being most of them elevated in direct proportion to adiposity, except adiponectin[3]. Adiponectin, leptin and ghrelin are involved in energy balance, regulating feeding behaviour[4]. Despite ghrelin being mainly produced in the stomach, visceral adipose tissue also secretes this hormone[5]. Enhanced activities of TNF-α and IL-6 are involved in the development of obesity-related insulin resistance, and PAI-1 in the impairment of fibrinolysis[6]. Other adipokines, like adiponectin and leptin, mitigate insulin resistance as they stimulate β-oxidation of fatty acids in skeletal muscle[4]. The understanding of adipokines molecular actions may lead to effective therapeutic strategies, ultimately designed to protect obese individuals against CVD, hypertension and type 2 diabetes.

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid, *cis*-9,*cis*-12-18 : 2 (*c*9,*c*12-18 : 2), with conjugated double bonds, which modulates

adiposity and related adipokine levels[7]. CLA is naturally found in foods of ruminant origin (e.g. as beef, lamb and dairy products) and is composed mainly by the *cis*-9, *trans*-11-18 : 2 (*c9,t11*-CLA) isomer, with the *trans*-10, *cis*-12-18 : 2 (*t10,c12*-CLA) isomer comprising only a small percentage[8]. It is well established that CLA has important effects on glucose and lipid metabolisms and that, especially the *t10,c12*-CLA isomer, is able to reduce fat deposition[9,10]. However, it has been reported that CLA, as a mixture of both isomers or its *t10,c12*-CLA isomer, induces potential side effects, such as hyperinsulinaemia and insulin resistance, promoting hepatic steatosis in lean and obese mouse models[11,12].

Additionally to CLA's adipokine modulation, other dietary fatty acids may interfere with adipokines expression and concentration[13,14]. Nevertheless, the relationship between CLA, adipokines and fatty acids in adipose tissue is far from clear in obese insulin-resistant models. Mutations affecting leptin action are associated with massive obesity in both human subjects and rodents[15]. For this purpose, the present study selected the *fa/fa* Zucker rat as experimental model, which develops morbid obesity due to the *fa* mutation on the leptin receptor. These rats display mild hyperglycaemia, pronounced hyperinsulinaemia, marked reduction in insulin sensitivity and hepatic steatosis[16].

Dietary manipulations, involving protein type and fat levels, result in complicated interactions with the fat-reducing effect of CLA[17]. This approach might help to support the beneficial effects of CLA and minimise its possible unfavourable side effects. Western diets provide a dramatic dietary fat imbalance, characterised by high percentages of saturated fats, which are among the most important causes of human mortality in developed countries[18]. To the best of our knowledge, the combination of dietary saturated fats from vegetable and animal origins with CLA effects has not been evaluated so far.

Facing this scenario, the main goal of the present study was to search for CLA and saturated fat diets effects on the serum adipokine profile of obese Zucker rats. High fat diets were formulated as atherogenic (with 2 % of cholesterol) based on two distinct saturated fats (palm oil or ovine fat) alone or combined with 1 % of CLA. Moreover, a principal component (PC) analysis was used in order to elucidate possible associations between the levels of individual fatty acids from adipose tissues and serum metabolites.

Materials and methods

Diet ingredients

All dietary components, except CLA oil and ovine fat, were purchased from Provimi Kliba SA (Kaiseraugst, Switzerland), which prepared and pelleted the experimental diets. The CLA oil (80 % purity) was a generous gift of PharmaNutrients Inc. (Gurnee, IL, USA) and contained a 1:1 mixture of *c9,t11* and *t10,c12*-CLA isomers. The ovine intraperitoneal fat was obtained from lambs fed with pelleted dehydrated lucerne supplemented with 6 % of a blend of sunflower and linseed oils[19]. The raw ovine fat was melted, and then filtered to subsequent incorporation in the diets.

Animals and diets

Experimental procedures were reviewed by the Ethics Commission of CIISA/FMV and approved by the Animal Care Committee of the National Veterinary Authority (Direcção-Geral de Veterinária, Portugal), following the appropriated European Union guidelines (N. 86/609/EEC). Obese male Zucker rats (*n* 32, Harlan Interfauna Iberia, Barcelona, Spain), ageing 5 weeks old, were individually housed in cages and maintained on a 12 h light–dark cycle at $22 \pm 2°C$. All rats had free access to tap water and semi-purified atherogenic diets, based on the AIN-93G formulation. Ingredients composition (% feed) was casein (20·0), dextrose (13·2), sucrose (11·9), maize starch (29·3), cellulose (5·0), AIN-93G vitamin mix (0·5), AIN-93G mineral mix (2·4), amino acids (0·3), cholesterol (2·0), cholic acid sodium salt (0·5) and butylated hydroxytoluene (0·01). After an acclimatisation period of 1 week, eight rats were allocated to one of the following dietary treatments: group palm oil, 11·3 % of palm oil plus 3·8 % sunflower oil; group palm oil diet + 1 % of CLA (PCLA), 11·3 % of palm oil plus 2·5 % sunflower oil plus 1·2 % CLA; group ovine fat, 11·3 % ovine fat plus 3·8 % sunflower oil; group ovine fat diet + 1 % of CLA (OCLA), 11·3 % ovine fat plus 2·5 % sunflower oil plus 1·2 % CLA. The proximate composition and fatty acid profile of the diets are shown in Table 1. Body weight and feed intake were recorded twice a week. After 14 weeks, rats were fasted for 12 h and killed by decapitation under light isoflurane anaesthesia. The trunk blood was centrifuged (1500 *g* for 10 min, at room temperature) to separate serum. Following blood collection, the organs were removed, weighted and stored at −80°C.

Serum biochemical assays

Total cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, glucose, total proteins, aspartate aminotransferase (AST, *EC* 2.6.1.1), alanine aminotransferase (*EC* 2.6.1.2) and alkaline phosphatase (*EC* 3.1.3.1) were analysed in serum through

Table 1. Composition and fatty acid profile of the diets

	P	PCLA	O	OCLA
Proximate composition (% DM)				
Crude protein	18	18	18	18
Crude fat	15	15	15	15
Crude ash	3·5	3·5	3·5	3·5
Crude fibre	3·5	3·5	3·5	3·5
Nitrogen-free extract	50	50	50	50
Fatty acid composition (% of total FAME)				
14 : 0	0·92	1·00	1·35	1·34
16 : 0	35·4	37·5	12·6	12·3
18 : 0	4·23	4·08	21·0	21·5
c9-18 : 1	34·9	32·3	22·3	21·0
t11-18 : 1	0·06	0·06	9·22	9·42
18 : 2*n*-6	20·0	16·0	15·7	12·2
18 : 3*n*-3	0·14	0·11	1·23	1·24
c9,t11-CLA	0·02	2·48	1·32	3·51
t10,c12-CLA	0·01	2·47	ND	2·10
Others	4·38	4·00	15·3	15·4

Dietary treatments: P, palm oil diet; PCLA, palm oil diet + 1 % of conjugated linoleic acid; O, ovine fat diet; OCLA, ovine fat diet + 1 % of conjugated linoleic acid; ND, not detected; FAME, fatty acid methyl esters; CLA, conjugated linoleic acid.

diagnostic test kits (Roche Diagnostics, Mannheim, Germany), using a Modular Hitachi Analytical System (Roche Diagnostics). VLDL-cholesterol and total lipids were calculated according to Friedewald et al. [20] and Covaci et al. [21] formulas, respectively.

Insulin, leptin, IL-1β, IL-6, TNF-α, monocyte chemoattractant protein-1 and total PAI-1 concentrations in serum were determined simultaneously through the Rat Serum Adipokine LINCOplex kit (RADPK-81K, Linco Research, Millipore, MA, USA), using the Luminex xMAP technology (Lincoplex 200, Linco Research). Adiponectin (EZRADP-62K, Linco Research), ghrelin (EZRGRT-91K, Linco Research) and CRP (CYT 294, Chemicon International, Millipore) levels were measured using commercial ELISA kits.

The degree of insulin resistance was calculated by the homeostasis model assessment using the insulin resistance index (HOMA-IR)[22]: fasting serum glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22·5. Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate high insulin resistance.

Hepatic lipid extraction

After liver lyophilisation, total lipids were extracted in duplicate, and gravimetrically measured using the procedure described by Fritsche et al. [23]. Briefly, lipids were extracted three times with methylene chloride–methanol (4:1 v/v) and a fourth time with n-hexane. Following evaporation and dry, the fatty residue was weighted.

Fatty acid composition of adipose tissues

Fatty acids methyl esters from epididymal and retroperitoneal adipose tissues were obtained using the method of Christie et al. [24], slightly modified by Raes et al. [25]. In short, 1 ml dry toluene was added to 0·05 g of lyophilised samples, and fatty acids were methylated through a base catalysis followed by an acid catalysis. At 50°C, sodium methoxide in anhydrous methanol (0·5 mol/l) reacted for 30 min, followed by HCl in methanol (1:1 v/v) for 10 min. fatty acids methyl esters were extracted twice with 3 ml n-hexane, and pooled extracts were evaporated until 2 ml volume under a stream of nitrogen. The resulting fatty acids methyl esters were then analysed by GC, using a fused-silica capillary column (CP-Sil 88; 100 m × 0·25 mm inner diameter × 0·20 mm film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA), equipped with a flame ionisation detector. The chromatographic conditions were described in detail by Jerónimo et al. [19]. Fatty acid composition was expressed as g/100 g of total fatty acids identified. The fatty acid composition of diets was obtained following the same analytical procedure described for adipose tissues.

Statistical analysis

Statistical analysis was carried out with the Statistical Analysis Systems software package, version 9.1 (SAS Institute, Cary, NC, USA). All data were reported as means with their standard errors. The proc GLM procedure was used to perform a 2 × 2 factorial analysis to determine significant main effects of CLA, fat source and their interaction (CLA × fat). When the interaction effect was significant, differences between groups were calculated using Tukey's post hoc test at $P < 0.05$.

In order to evaluate differences between fatty acid composition from epididymal and retroperitoneal adipose tissues, the proc GLM procedure was used with tissue as a single factor. Afterwards, PC analysis was performed, using proc PRINCOMP of SAS, to assess relationships between fatty acids (average between fatty acids from epididymal and retroperitoneal adipose tissues) and adipokines. After data normalisation, the analysis was based on the correlation matrix (consisting of twenty-nine variables) and PC were considered as significant if they contributed more than 5 % for the total variance.

Results

Body composition

The daily feed intake and body weight, as well as both adipose tissues and liver weights, are presented in Table 2. CLA-fed rats had lower final body weight due, in part, to a decrease in the average daily intake induced by CLA ($P < 0.01$). In the same manner, liver weight and hepatic lipids were reduced in PCLA and OCLA groups in relation to their matching groups ($P < 0.001$). In contrast, neither CLA nor fat source had any effect on the weight of epididymal and retroperitoneal adipose tissues ($P > 0.05$). The interaction CLA × fat was not observed for any of those parameters ($P > 0.05$). Finally, the weight of longissimus dorsi muscle, kidney, testicle, spleen, heart and lung did not present statistical differences ($P > 0.05$, data not shown).

Serum metabolite profile

The serum metabolite profile is also listed on Table 2. Animals fed CLA had higher levels of total and LDL-cholesterol ($P < 0.05$) and lower glucose levels ($P < 0.05$). Insulin and HOMA-IR were higher in ovine fat groups compared with palm oil groups ($P < 0.05$), but were not affected by CLA ($P > 0.05$). Regarding the hepatic enzymes, lower circulating AST concentrations were found for CLA-supplemented animals ($P < 0.05$). CLA enhanced adiponectin levels ($P < 0.001$) regardless the fat source. Leptin levels were affected by the fat source but not by the supplementation with CLA, being higher in ovine fat groups ($P < 0.001$). Neither CLA nor fat source had effects on ghrelin concentrations ($P > 0.05$). The changes in the pro-inflammatory markers IL-1β, IL-6, TNF-α and CRP are also summarised on Table 2. IL-1β and TNF-α were affected by the fat source independently of CLA supplementation, being higher in ovine fat groups ($P < 0.05$). IL-6 was dependent on the interaction between CLA and fat source. OCLA group presented higher values of IL-6 than the other dietary treatments ($P < 0.05$). CRP and monocyte chemoattractant protein-1 levels were similar for all dietary groups ($P > 0.05$). In contrast, CLA reduced PAI-1 concentrations regardless the fat source ($P < 0.01$).

Table 2. Body composition variables and serum metabolites

	P	PCLA	O	OCLA	SEM	CLA	Fat	CLA × fat
						Significance level		
Growth and body composition								
Initial body weight (g)	240	234	234	234	11·2	NS	NS	NS
Final body weight (g)	577	552	584	511	12·7	***	NS	NS
Daily feed intake (g/d)	23·7	23·0	23·9	21·8	0·508	**	NS	NS
Retroperitoneal fat weight (g)	24·0	24·7	23·7	20·7	1·62	NS	NS	NS
Epididymal fat weight (g)	13·7	15·2	14·7	14·6	0·605	NS	NS	NS
Liver weight (g)	62·2	54·7	60·6	48·1	1·88	***	*	NS
Hepatic lipids (% liver weight)	21·9	18·9	23·5	19·2	0·800	***	NS	NS
Serum biochemistry profile								
Total cholesterol (mg/l)	11 719	15 173	13 705	16 271	1333	*	NS	NS
HDL-cholesterol (mg/l)	1811	1759	1901	1849	101	NS	NS	NS
LDL-cholesterol (mg/l)	7485	10 001	9205	10 526	876	*	NS	NS
VLDL-cholesterol (mg/l)†	885	1211	870	1061	169	NS	NS	NS
TAG (mg/l)	4425	6068	4373	5299	844	NS	NS	NS
Total lipids (mg/l)‡	20 490	26 543	23 557	26 751	2421	NS	NS	NS
Total proteins (g/l)	78·7	71·7	75·8	70·2	3·26	NS	NS	NS
Glucose (mg/l)	1155	1061	1226	1076	56·6	*	NS	NS
Insulin (ng/ml)	1·78	1·84	3·02	2·53	0·26	NS	**	NS
HOMA-IR (mmol/l × mU/l)§	14·1	14·4	25·5	18·4	2·68	NS	*	NS
Serum hepatic markers								
AST (U/l)	355	287	341	240	37·7	*	NS	NS
ALT (U/l)	69·4	85·9	78·0	88·9	6·93	NS	NS	NS
ALP (U/l)	284	283	226	237	33·2	NS	NS	NS
Serum adipokine profile								
Adiponectin (μg/ml)	11·6	15·3	10·9	14·6	0·716	***	NS	NS
Leptin (ng/ml)	3·61	3·03	6·22	5·48	0·688	NS	***	NS
Ghrelin (ng/ml)	1·22	1·06	0·75	1·06	0·184	NS	NS	NS
IL-1β (pg/ml)	175	196	451	448	49·4	NS	***	NS
IL-6 (pg/ml)	165[b]	148[b]	179[b]	259[a]	20·5	NS	**	*
TNF-α (pg/ml)	24·1	24·4	24·9	25·7	0·411	NS	*	NS
CRP (pg/ml)	250	320	286	292	33·7	NS	NS	NS
MCP-1 (pg/ml)	561	455	371	588	100	NS	NS	NS
PAI-1 (pg/ml)	370	243	560	202	77·0	**	NS	NS

Dietary treatments: P, palm oil diet; PCLA, palm oil diet + 1 % of conjugated linoleic acid; O, ovine fat diet; OCLA, ovine fat diet + 1 % of conjugated linoleic acid.
AST, aspartate aminotransferase (EC 2.6.1.1); ALT, alanine aminotransferase (EC 2.6.1.2); ALP, alkaline phosphatase (EC 3.1.3.1); CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.
Mean values were significant: NS, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[a,b] Mean values within the same row with different superscript letters are statistically different (Tukey's *post hoc* test, $P < 0.05$).
† VLDL-cholesterol = 1/5 (TAG)
‡ Total lipids = (total cholesterol) × 1·12 + (TAG) × 1·33 + 148.
§ HOMA-IR, insulin resistance index = (fasting serum glucose) × (fasting serum insulin)/22·5.

Fatty acid composition of epididymal and retroperitoneal adipose tissues

The fatty acid composition of epididymal and retroperitoneal adipose tissues are presented in Tables 3 and 4, respectively. In both tissues, the effect of fat source was detected for all fatty acids ($P < 0.001$). Palm oil groups were richer in 16 : 0, c9-16 : 1, c9-18 : 1, 18 : 2n-6 and 20 : 4n-6 fatty acids than ovine fat groups ($P < 0.001$). Rats fed ovine fat presented higher values of 14 : 0, c7-16 : 1, 18 : 0, t10 + t11-18 : 1, 18 : 3n-3 and CLA isomers than groups fed palm oil ($P < 0.001$). CLA-fed rats had more c7-16 : 1 and t10 + t11-18 : 1, and less c9-18 : 1, 18 : 2n-6 and 20: 4n-6 fatty acids ($P < 0.001$). Moreover, CLA supplementation increased the percentages of c9,t11-CLA comparing with their matching groups ($P < 0.001$) in both adipose tissues. Also, t10, c12-CLA isomer and the other CLA isomers were increased in PCLA and OCLA groups ($P < 0.001$), but in a lower magnitude. About fatty acids sums, CLA had no effect on total SFA ($P > 0.05$) from epididymal adipose tissue, but increased it in retroperitoneal adipose tissue ($P < 0.05$).

Total MUFA and total PUFA had lower percentages in CLA-fed rats ($P < 0.001$). In both tissues, the Δ9-indices were affected by CLA × fat interaction ($P < 0.05$). The Δ9-index16 ratio was higher in O group than the others ($P < 0.05$), and palm oil and PCLA groups had higher Δ9-index18 ratio than ovine fat groups ($P < 0.05$). As shown in Tables 3 and 4, the response of fatty acid composition in epididymal adipose tissue had a similar pattern as the one described for retroperitoneal adipose tissue. This fact was confirmed by the ANOVA with a single factor. Only c9-16 : 1 and c11-18 : 1 fatty acids had differences between adipose tissues ($P < 0.001$; data not shown). Due to this similarity, the subsequent PC statistical analysis was performed using the average values of fatty acids from epididymal and retroperitoneal adipose tissues.

Principal component analyses

The results of PC analysis of fatty acid composition of adipose tissues and serum metabolites are shown in Table 5. The five

Table 3. Fatty acid composition (% of total fatty acids methyl esters (FAME)) of epididymal adipose tissue

	P	PCLA	O	OCLA	SEM	Significance level		
						CLA	Fat	CLA × fat
14:0	1.05	1.24	1.30	1.42	0.020	***	***	NS
16:0	25.2	25.69	18.80	19.00	0.167	NS	***	NS
c7-16:1	0.42	0.51	0.52	0.66	0.013	***	***	NS
c9-16:1	7.59[a]	7.87[a]	6.67[b]	6.18[c]	0.111	NS	***	**
18:0	1.98[b]	1.77[b]	4.19[a]	4.27[a]	0.065	NS	***	*
c9-18:1	40.9	39.4	36.5	35.3	0.198	***	***	NS
c11-18:1	4.67[b]	4.91[a]	4.75[b]	4.59[b]	0.052	NS	**	***
t10 + t11-18:1	ND	0.31	3.30	3.53	0.044	***	***	NS
18:2n-6	15.3	13.3	13.8	12.2	0.155	***	***	NS
18:3n-3	0.17	0.16	0.77	0.74	0.014	NS	***	NS
c9,t11-CLA	ND	1.55[c]	2.37[b]	4.09[a]	0.038	***	***	*
t10,c12-CLA	ND	0.58	0.15	0.72	0.016	***	***	NS
CLA others	ND	0.03[b]	ND	0.26[a]	0.005	***	***	***
20:4n-6	0.67	0.31	0.56	0.25	0.017	***	***	NS
Total FAME	98.1	97.6	93.7	93.3	0.079	***	***	NS
Others	1.95	2.45	6.33	6.72	0.079	***	***	NS
SFA†	28.3	28.7	24.3	24.7	0.206	NS	***	NS
MUFA‡	53.6	52.7	48.4	46.8	0.216	***	***	NS
PUFA§	16.2	13.7	15.2	13.2	0.162	***	***	NS
Total CLA‖	ND	2.16[c]	2.51[b]	5.07[a]	0.051	***	***	***
Δ9-index16¶	1.30[b]	1.31[b]	1.36[a]	1.33[b]	0.007	NS	***	*
Δ9-index18††	21.8[b]	23.4[a]	9.74[c]	9.28[c]	0.356	NS	***	*

Dietary treatments: P, palm oil diet; PCLA, palm oil diet + 1% of conjugated linoleic acid; O, ovine fat diet; OCLA, ovine fat diet + 1% of conjugated linoleic acid. ND, not detected.

Mean values were significant: NS, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[a,b,c] Mean values within the same row with different superscript letters are statistically different (Tukey's *post hoc* test, $P < 0.05$).

† SFA = sum of 14:0, 16:0 and 18:0.

‡ MUFA = sum of c7-16:1, c9-16:1, c9-18:1 and c11-18:1

§ PUFA = sum of 18:2n-6, 18:3n-3 and 20:4n-6.

‖ Total CLA = sum of c9,t11-CLA, t10,c12-CLA and CLA others

¶ Δ9-index16, desaturation index 16:0 to 16:1 = 16:0/(16:0 + c9-16:1).

†† Δ9-index18, desaturation index 18:0 to 18:1 = 18:0/(18:0 + c9-18:1).

first PC explained 87·56% of the total variance. The first and second PC were responsible for 63·48% of the total variance, 43·85 and 19·63%, respectively. The PC3 explained 10·08%, the PC4 7·64% and the PC5 6·36% of the variability. As total variance explained by the first two PC is greater than 50%, the projection of fatty acids and serum metabolites in the plane defined by these PC is shown in Fig. 1. Overall, PC1 was mainly characterised by 16:0 (0·84), c9-16:1 (0·88) and c9-18:1 (0·97) fatty acids on the right side and by 14:0 (−0·83), c7-16:1 (−0·94), 18:0 (−0·91), t10 + t11-18:1 (−0·94), c9,t11-CLA (−0·97) and 18:3n-3 (−0·88) fatty acids on the left side. The fatty acids 16:0 and c9-16:1, mainly found in palm oil diets, are in opposition to 18:0 and 18:1 *trans* fatty acids, mainly found in ovine fat diets (Table 1). Therefore, PC1 discriminated between fatty acids associated with palm oil and ovine fat added to the diets. The PC2 clearly distinguished t10,c12-CLA isomer (0·71) and adiponectin (0·71), located in the upper part, from PAI-1 (−0·76) and 20:4n-6 (−0·68), located in the lower part of the graphic. As c9,t11-CLA isomer was already present in the ovine fat diets and the only source of t10,c12-CLA isomer was the supplementation, the PC2 discriminated between CLA supplementation and no CLA supplementation. In quadrant A, a group was defined by c9,t11-CLA isomer, other CLA isomers, 14:0, c7-16:1 and TNF-α. In quadrant B, another group was distinguished, composed by c9-16:1, c9-18:1, c11-18:1 and ghrelin. The fatty acids 18:0, 18:3n-3 and t10 + t11-18:1, which showed enhanced

values in ovine fat-fed animals, appeared grouped in quadrant D and highly correlated with leptin, IL-1β and insulin. Interestingly, this group of adipokines was opposed to 16:0; the t10,c12-CLA isomer was opposed to 20:4n-6 and PAI-1; and the c9,t11-CLA isomer opposed to TAG. The variables with higher loadings were HDL-cholesterol (−0·74), IL-6 (−0·68) and CRP (0·71) in PC3; total-cholesterol (−0·63), TAG (−0·65) and glucose (0·63) in PC4; and adiponectin (−0·48) in PC5.

Discussion

The present investigation showed that animals fed CLA, regardless the fat source, gained less body weight, in parallel with a reduction in liver weight and hepatic lipids content. A decrease in daily feed intake by CLA was also observed, yet this variation seems insufficient to fully explain the variations in final body weight. Taking into account the conversion of food ingested on the weight gain, the average was 7·0 and 7·2 g food/g weight for palm oil and ovine fat groups. As the difference in feed intake was 68·6 and 205·8 g for palm oil diet/PCLA and ovine fat diet/OCLA groups, respectively, only 9·8 and 28·6 g of the total weight gain is explained by the food, remaining to justify 15·2 and 44·4 g. In view of the fact that no other tissues weight were affected (though subcutaneous fat was not collected due to isolation difficulties), liver was likely the organ responsible for the less weight gain. It was also observed that the decrease

Table 4. Fatty acid composition (% of total fatty acids methyl esters (FAME)) of retroperitoneal adipose tissue

	P	PCLA	O	OCLA	SEM	CLA	Fat	CLA × fat
						Significance level		
14:0	1·07^c	1·27^b	1·40^a	1·48^a	0·024	***	***	*
16:0	25·1	25·8	18·1	18·4	0·180	**	***	NS
c7-16:1	0·40	0·52	0·55	0·70	0·009	***	***	NS
c9-16:1	6·81^a	6·80^a	6·09^b	5·37^c	0·114	**	***	**
18:0	1·95^b	1·72^b	4·35^a	4·38^a	0·061	NS	***	*
c9-18:1	40·0	39·1	35·8	35·0	0·129	***	***	NS
c11-18:1	5·91	6·01	5·37	5·46	0·096	NS	***	NS
t10 + t11-18:1	ND	0·29	3·64	3·78	0·046	***	***	NS
18:2n-6	15·6^a	13·1^c	13·9^b	12·2^d	0·134	***	***	**
18:3n-3	0·12	0·10	0·73	0·69	0·010	**	***	NS
c9,t11-CLA	0·05^d	1·70^c	2·51^b	4·39^a	0·032	***	***	**
t10,c12-CLA	ND	0·62	0·16	0·75	0·018	***	***	NS
CLA others	ND	0·05^b	ND	0·32^a	0·009	***	***	***
20:4n-6	0·73^a	0·29^c	0·62^b	0·27^c	0·017	***	***	*
Total FAME	97·7	97·4	93·2	93·1	0·129	NS	***	NS
Others	2·34	2·65	6·83	6·89	0·129	NS	***	NS
SFA†	28·1	28·8	23·8	24·2	0·211	*	***	NS
MUFA‡	53·1	52·4	47·8	46·5	0·162	***	***	NS
PUFA§	16·4^a	13·5^c	15·3^b	13·2^c	0·138	***	***	**
Total CLA‖	0·05^d	2·38^c	2·67^b	5·46^a	0·042	***	***	***
Δ9-index16¶	1·27^{bc}	1·26^c	1·34^a	1·29^b	0·006	***	***	**
Δ9-index18††	21·5^b	23·7^a	9·25^c	8·98^c	0·330	**	***	***

Dietary treatments: P, palm oil diet; PCLA, palm oil diet + 1 % of conjugated linoleic acid; O, ovine fat diet; OCLA, ovine fat diet + 1 % of conjugated linoleic acid. ND, not detected.
Mean values were significant: NS, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
^{a,b,c,d} Mean values within the same row with different superscript letters are statistically different (Tukey's *post hoc* test, $P < 0.05$).
† SFA = sum of 14:0, 16:0 and 18:0.
‡ MUFA = sum of c7-16:1, c9-16:1, c9-18:1 and c11-18:1.
§ PUFA = sum of 18:2n-6, 18:3n-3 and 20:4n-6.
‖ Total CLA = sum of c9,t11-CLA, t10,c12-CLA and CLA others.
¶ Δ9-index16, desaturation index 16:0 to 16:1 = 16:0/(16:0 + c9-16:1).
†† Δ9-index18, desaturation index 18:0 to 18:1 = 18:0/(18:0 + c9-18:1).

in feed intake by CLA was not mediated by ghrelin. In agreement with the present findings, other authors[26–29] have reported a reduction in hepatic lipids content by CLA and similar adipose tissue weights.

Some authors reported CLA beneficial effects in obese Zucker rats by increasing plasma adiponectin levels and, therefore, alleviating hyperinsulinaemia[26,30]. Indeed, in the present study, CLA increased adiponectin and decreased glucose levels without affecting insulin homeostasis. Instead, higher insulin and HOMA-IR values were found in rats fed ovine fat compared with rats fed palm oil. In this regard, the fat source effect in ovine fat diets, by rising up insulin levels, could overlap CLA reducing glucose effects. Also, independently of CLA supplementation, ovine fat diets increased the inflammatory markers TNF-α and IL-1β, although IL-6 only increased in the OCLA group. These inflammatory changes in ovine fat groups appeared insufficient to increase CRP and monocyte chemoattractant protein-1 serum levels. In the present study, CLA had no effects on leptin levels, but ovine fat-based diets increased this adipokine. In contrast, Noto *et al.*[30] and Halade *et al.*[12] observed the ability of CLA to reduce leptin in serum.

Alessi *et al.*[31] reported that plasma levels of PAI-1 are closely related to the degree of liver steatosis than to the fat accumulation in adipose tissue. In the present work, PAI-1 showed lower levels in CLA-supplemented groups, which possibly resulted from a decrease in hepatic lipids content. Serum levels of AST and alanine aminotransferase are normally increased in steatotic liver cases, and a stronger correlation between PAI-1 and AST rather than with alanine aminotransferase was reported by Alessi *et al.*[31]. In fact, our animals fed with CLA had a concomitant decrease in AST and PAI-1.

The administration of atherogenic diets allowed us to exploit if CLA is able to reverse the adverse affects of cholesterol, namely, in serum lipid profile and attenuating hepatic steatosis. Regarding the content of cholesterol in lipoproteins, CLA reduced VLDL and LDL fractions as well as total cholesterol in obese Zucker rats[27]. However, CLA induced the development of aortic fatty streaks in C57BL/6J mice[32]. Surprisingly, in the present study, CLA increased total and LDL-cholesterol without affecting serum TAG. The changes found for cholesterol profile and PAI-1 led us to look for possible alterations in aorta microvasculature. The histological examination of different transversal segments of those aortas revealed only small morphological changes affecting smooth muscle cells, but no atheroma plaques were observed for any dietary group (images not shown).

The fatty acid composition of epididymal and retroperitoneal adipose tissues showed similar patterns. CLA induced an increase in c7-16:1 and t10 + t11-18:1 and a decrease in c9-18:1, 18:2n-6 and 20:4n-6 fatty acids. Sunflower oil

Table 5. Loadings for the first five principal components (PC)

| Variables	PC1	PC2	PC3	PC4	PC5
14:0	−0.83*	0.22	0.42	−0.09	−0.04
16:0	0.84*	0.49	−0.02	0.09	−0.11
c7-16:1	−0.94*	0.23	0.02	−0.18	−0.09
c9-16:1	0.88*	0.16	0.12	−0.27	−0.20
18:0	−0.91*	−0.36	0.00	0.06	0.13
c9-18:1	0.97*	0.12	−0.13	−0.01	−0.06
c11-18:1	0.74	−0.04	0.12	−0.22	−0.29
t10 + t11-18:1	−0.94*	−0.29	0.08	−0.01	0.10
18:2n-6	0.75	−0.56	−0.27	0.16	0.04
18:3n-3	−0.88*	−0.43	0.05	0.02	0.12
c9,t11-CLA	−0.97*	0.18	0.11	−0.09	0.01
t10,c12-CLA	−0.65	0.71*	0.13	−0.13	−0.09
CLA others	−0.77	0.40	−0.33	−0.10	0.00
20:4n-6	0.63	−0.68*	−0.21	0.17	0.04
Total cholesterol	−0.10	−0.59	−0.10	−0.63*	−0.24
HDL-cholesterol	−0.32	−0.01	−0.74*	0.14	−0.35
LDL-cholesterol	−0.15	−0.60	−0.18	−0.49	−0.42
TAG	0.40	−0.05	0.44	−0.65*	0.45
Glucose	−0.01	−0.38	0.27	0.63*	−0.54
Insulin	−0.50*	−0.57*	0.27	−0.11	−0.37
Adiponectin	−0.23	0.71	0.13	−0.13	−0.48*
Leptin	−0.75*	−0.61	0.17	0.04	0.08
Ghrelin	0.63*	0.02	−0.04	−0.36	0.27
IL-1β	−0.77*	−0.43	−0.03	0.18	0.17
IL-6	−0.31	−0.15	−0.68*	−0.53	−0.17
TNF-α	−0.67*	0.36	−0.52	0.04	0.03
CRP	−0.10	0.38	0.71*	−0.06	−0.39
MCP-1	−0.02	0.57	−0.43	0.02	−0.20
PAI-1	0.30	−0.76*	0.21	−0.04	−0.32
Portion of variance (%)	43.85	19.63	10.08	7.64	6.36
Cumulative variance (%)	43.85	63.48	73.56	81.2	87.56

CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

* The most significant loadings.

(rich in 18:2n-6) replaced CLA oil in the diets without supplementation, and so a reduction of 18:2n-6 in CLA-supplemented groups was expected. This decrease probably also explain the reduction in 20:4n-6 levels, an endogenous metabolite of 18:2n-6. The incorporation of CLA isomers into adipose tissues did not reflect the CLA profile of dietary treatments, being the t10,c12-CLA less incorporated than the c9,t11-CLA isomer, as observed by other authors[33]. Apart from a possible endogenous synthesis in the rat, the c9,t11-CLA levels in adipose tissue were mostly provided by the ovine fat-enriched diet. Park et al. [34] reported a clear decrease in stearoyl-CoA desaturase enzyme activity by CLA. In both adipose tissues, it was not observed a clear effect of CLA on Δ9-indices (a proxy for stearoyl-CoA desaturase capability), but a CLA × fat interaction, meaning that CLA action depends on the fatty acid profile available in the diet.

In addition to the CLA ability to modulate adipokine metabolism affecting glucose and lipid metabolisms, namely, insulin sensitivity, other subgroups of fatty acids are capable of influencing these metabolisms, as well. In rats with hyper-leptinaemia and hyperinsulinaemia, dietary n-3 PUFA caused a 40–50 % reduction in plasma levels of leptin and insulin[13]. A recent work has demonstrated that 16:0 and c9-18:1 inhibited hepatic insulin signalling in hepatocyte cell cultures[14]. Nevertheless, there is insufficient knowledge about how fatty acid profiles of adipose tissue are correlated with serum metabolites concentration. In the present study, a PCA was carried out to explore the relationships between the deposition of individual fatty acids in adipose tissue and serum metabolites. The projection of variables in the PC1 × PC2 plane (Fig. 1), explaining a substantial percentage of the total variance (63 %), revealed some interesting associations. Fatty acids distribution in the graph can be fairly interpreted by its common dietary origin, with quadrants A and D related with saturated fat source and quadrants A and B with CLA supplementation. Adiponectin was located close to the t10,c12-CLA isomer and, on the opposite, PAI-1, which in turn was near 20:4n-6 and its precursor 18:2n-6. Indeed, a study in human subjects showed that dietary 18:2n-6 and 20:4n-6 were positively associated with PAI-1 activity[35]. In relation to c9,t11-CLA, this isomer appeared inversely related to TAG, which corroborates the reduction on plasma TAG levels in obese rodents fed this isomer[36,37]. TNF-α was located near the 14:0, c7-16:1 and c9,t11-CLA in the lower region of quadrant A, reflecting the influence of both ovine fat and CLA supplementation. Leptin and insulin were located in left region of quadrant D, together with 18:0, t10 + t11-18:1, 18:3n-3, suggesting that they are mainly related to fatty acids in ovine fat diets. Besides the fact that 16:0 has been associated with inflammation[38,39], in the present study, this predominant SFA in palm oil diets was located contrarily to IL-1β and IL-6. The location of ghrelin in the border of quadrants B and C showed that this hormone is
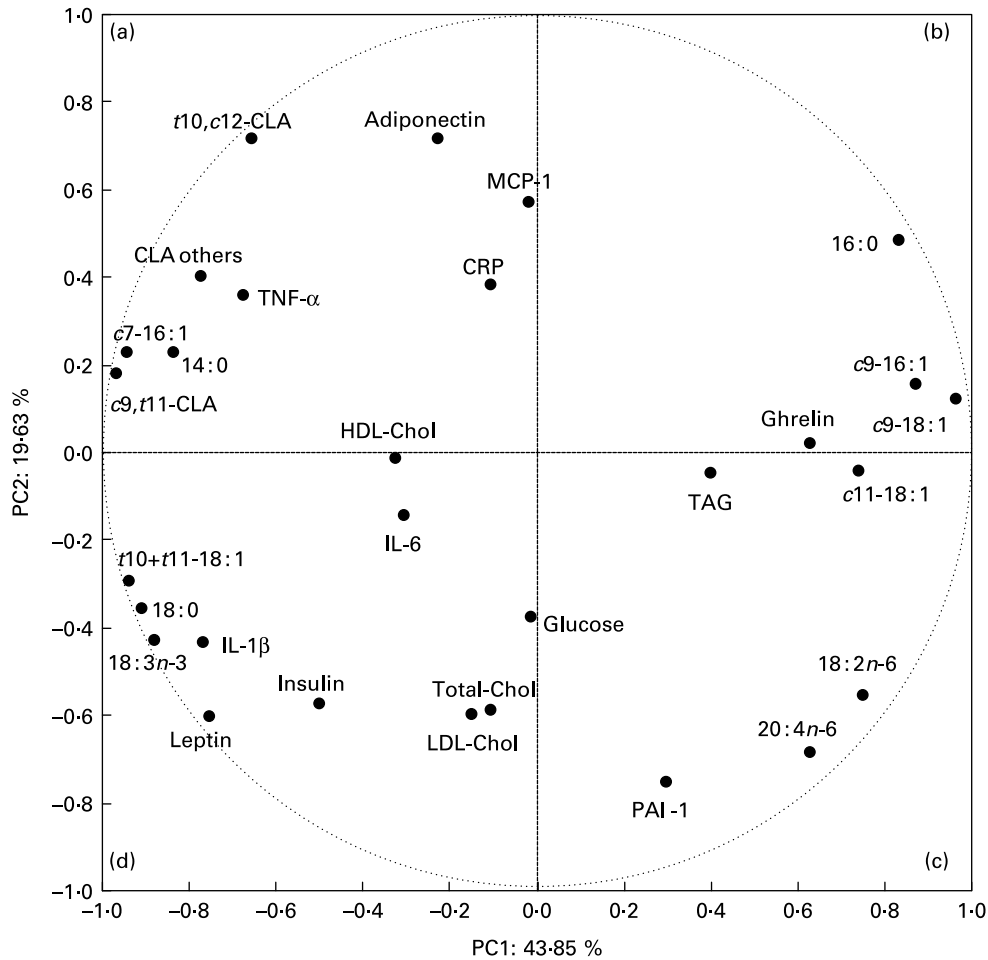
Fig. 1. Projection of the variables (average of fatty acids percentages from epididymal and retroperitoneal adipose tissues and contents of serum metabolites) in the plane defined by the two first principal components (PC1 and PC2 loadings plot). CLA, conjugated linoleic acid; *c*9,*t*11-CLA, *cis*-9,*trans*-11-CLA; *t*10,*c*12-CLA, *trans*-10,*cis*-12-CLA; CRP, C-reactive protein; HDL-Chol, HDL-cholesterol; LDL-Chol, LDL-cholesterol; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; Total-Chol, total-cholesterol.

mainly associated with dietary palm oil. The variables located near the origin of the PC1 and PC2 axis were not related with any of the studied factors. The associations between *t*10,*c*12-CLA, positively with adiponectin and negatively with PAI-1, reinforce our belief that *t*10,*c*12-CLA, but not *c*9,*t*11-CLA, is the main isomer responsible for CLA effects in the present work. As it is not possible to draw definite causal relationships with these exploratory techniques of data analysis, some associations should be exploited in well-directed experimental designs. For instance, the effects of 18:2*n*-6 and *c*9-18:1 on serum ghrelin concentrations should be further evaluated.

In the present study, the specific effects observed for ovine fat treatments may be attributed to the different fatty acid composition of the diets containing fat from vegetable or animal sources (Table 1). In concrete, the influence on insulin resistance and pro-inflammatory profile can be mainly due to the imbalance between the pro-inflammatory SFA (sum of 16:0 and 18:0, 40–42 *v.* 34 %, for vegetable and animal fat diets, respectively) and the anti-inflammatory MUFA (percentage of *c*9-18:1, 32–35 *v.* 21–22 %, for vegetable and animal fat diets, respectively) present in the diet[38]. Concerning *trans* fatty acids, *t*11-18:1 is the predominant in animal fats (9 % of total fatty acids methyl esters), and is

precursor of *c*9,*t*11-CLA by endogenous conversion in tissues[40]. In fact, it was recently reported that *t*11-18:1, in contrast to *t*10-18:1 isomer, is neutral or even beneficial to aortic lipid deposition in rabbits[41]. Moreover, ovine fat diets contain minor amounts of *trans* fatty acids derived from rumen biohydrogenation, *trans* and *cis* isomers of oleic acid and conjugated and non-conjugated *trans* isomers of linoleic acid, not discriminated in the present study, which can display additional roles on these effects.

Conclusions

The results suggest that CLA supplementation of diets rich in saturated fats and cholesterol has some beneficial effects in obese Zucker rats by increasing adiponectin and decreasing PAI-1 serum levels, as well as alleviating hepatic steatosis through hepatic lipids reduction. However, this decrease does not seem to be enough to improve insulin sensitivity. The multivariate analysis suggested that CLA effects observed on adiponectin and PAI-1 levels can be mainly attributed to the *t*10,*c*12-CLA isomer. On the other hand, diets enriched in ovine fat appear to promote an increase in serum concentrations of some pro-inflammatory cytokines, also increasing

insulin resistance. The present work questions the usefulness of CLA to prevent obesity in Western societies, whereas high intake of animal saturated fats is prevalent. Nonetheless, the combination of CLA and saturated fat diets from vegetable origin seems to attenuate prejudicial effects promoted by animal fats.

Acknowledgements

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