

## Distinct effects of oleic acid and its *trans*-isomer elaidic acid on the expression of myokines and adipokines in cell models

Nuria Granados, Jaume Amengual†, Joan Ribot, Andreu Palou\* and M. Luisa Bonet

Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears, CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERObn), Cra. Valldemossa, Km 7.5, 07122 Palma de Mallorca, Spain

(Received 3 June 2010 – Revised 25 October 2010 – Accepted 27 October 2010 – First published online 5 January 2011)

### Abstract

*Trans*-fatty acids (TFA) and *cis*-monounsaturated fat appear to exert detrimental and beneficial effects, respectively, on glucose metabolism and insulin sensitivity. Adipose tissue and skeletal muscle are a source of signalling proteins (adipokines and myokines), some of which have been related to the control of insulin sensitivity. Here, we investigated the possible differential effects of elaidic acid (EA; *trans*-9-18:1) – the major component in industrially produced TFA – and oleic acid (OA; *cis*-9-18:1) – its *cis*-isomer naturally present in food – on cellular glucose uptake and the expression of selected myokines and adipokines using cell models. Differentiated C2C12 myotubes and 3T3-L1 adipocytes were pretreated with the vehicle (control cells) or fatty acids for 24 h, after which basal and insulin-stimulated 2-deoxyglucose uptake and the expression of selected signalling proteins were measured. In C2C12 myotubes, pretreatment with OA, but not with EA, led to increased insulin-stimulated 2-deoxyglucose uptake and IL-6 expression levels, while pretreatment with EA, but not with OA, led to reduced IL-15 mRNA levels and increased TNF- $\alpha$  expression levels. In 3T3-L1 adipocytes, exposure to OA, but not to EA, resulted in reduced resistin gene expression and increased adiponectin gene expression. The results show evidence of distinct, direct effects of OA and EA on muscle glucose uptake and the expression of target myokines and adipokines, thus suggesting novel mechanisms by which *cis*- and *trans*-monounsaturated fat may differentially affect systemic functions.

**Key words:** Adipokines and myokines: Glucose uptake: *Trans*-fatty acids: Oleic acid: Cell models

*Trans*-fatty acids (TFA) are unsaturated fatty acids with at least one double bond in the *trans*, rather than the typical *cis*, configuration. A major source of TFA in food is industrially produced *trans*-fatty acids (IP-TFA) generated during partial hydrogenation ('hardening') of vegetable fat. Industrial hydrogenation involves heating the raw oils to about 400°C under high pressure and with the addition of different catalysts, and in this process, *trans*-double bonds are undesirably formed along with fatty acid molecules from position six and higher, at the same time as SFA are formed. Partially hydrogenated fat is used in a variety of food products, especially spreads, baked goods and fast food. Elaidic acid (EA; *trans*-9-18:1), the *trans*-isomer of oleic acid (OA), is the predominant IP-TFA<sup>(1,2)</sup>.

While there is evidence pointing to favourable effects of OA-enriched diets and of OA itself on CVD risk, weight maintenance and insulin sensitivity<sup>(3–5)</sup>, dietary TFA is generally associated with detrimental effects on health<sup>(2,6,7)</sup>. Importantly, deleterious effects are well

documented for IP-TFA, but not for TFA of ruminant origin, in the low amounts usually consumed<sup>(2,6)</sup>. In fact, health benefits have been suggested for conjugated linoleic acid and vaccenic acid (*trans*-11-18:1), the major TFA naturally present in beef and dairy products<sup>(8,9)</sup>.

Adverse effects of IP-TFA consumption on multiple cardiovascular risk factors are well established, including promotion of a pro-atherogenic serum lipid/lipoprotein profile, endothelial dysfunction and an increased production of pro-inflammatory cytokines<sup>(6,7,10)</sup>. The impact of IP-TFA consumption on insulin resistance and the risk of type 2 diabetes is more controversial, since detrimental effects have been reported in some, but not all, human observational studies and short-term intervention trials on the topic<sup>(11–14)</sup>. Likewise, in rats, the effects of TFA-enriched diets (fed for 8–12 weeks) increasing insulin resistance and impairing systemic and tissue-specific glucose disposal have been reported in some studies<sup>(15–17)</sup>, but not in others<sup>(18)</sup>. Nevertheless, in

**Abbreviations:** 2-DOG, 2-deoxy-D-glucose; EA, elaidic acid; FBS, fetal bovine serum; IP-TFA, industrially produced *trans*-fatty acid; OA, oleic acid; TFA, *trans*-fatty acids.

\* **Corresponding author:** Professor A. Palou, fax +34 971 173426, email andreu.palou@uib.es

† Present address: Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH, USA.

a long-term, 5-year controlled study in monkeys, TFA feeding (*v.* OA) caused insulin resistance and impaired glucose metabolism along with abdominal obesity<sup>(19)</sup>. Two long-term prospective observational studies in humans have also suggested that TFA consumption promotes weight gain, and particularly accumulation of abdominal fat<sup>(20,21)</sup>. Abdominal obesity, insulin resistance and CVD risk are intimately linked<sup>(22)</sup>.

Mechanisms underlying the relationship between dietary fatty acids and metabolic health are incompletely understood and seem to include the effects on cell-membrane fatty acid composition and function, as well as on gene expression and enzyme activity in key tissues<sup>(5)</sup>. Both adipose tissue and skeletal muscle produce and secrete signalling proteins (named adipokines and myokines, respectively), which may affect metabolic health, and particularly glucose metabolism and insulin sensitivity locally and systemically. Studies examining the possible differential, direct effects of concrete species of *cis*- and *trans*-MUFA on the cellular production of such signals are lacking, yet of potential interest to support the biological plausibility of observations derived from epidemiological studies and studies using complex mixtures. Therefore, the aim of the present study was to compare the direct effects of EA, the major IP-TFA, and its *cis*-isomer, OA, naturally present in foods on the expression of myokines and adipokines possibly involved in the control of insulin sensitivity, along with their effects on basal and insulin-stimulated glucose uptake, in muscle and adipocyte cell models.

## Experimental methods

### Cell culture and fatty acid treatment

All reagents used for cell culture and treatment including insulin from bovine pancreas (product no. I4011), OA

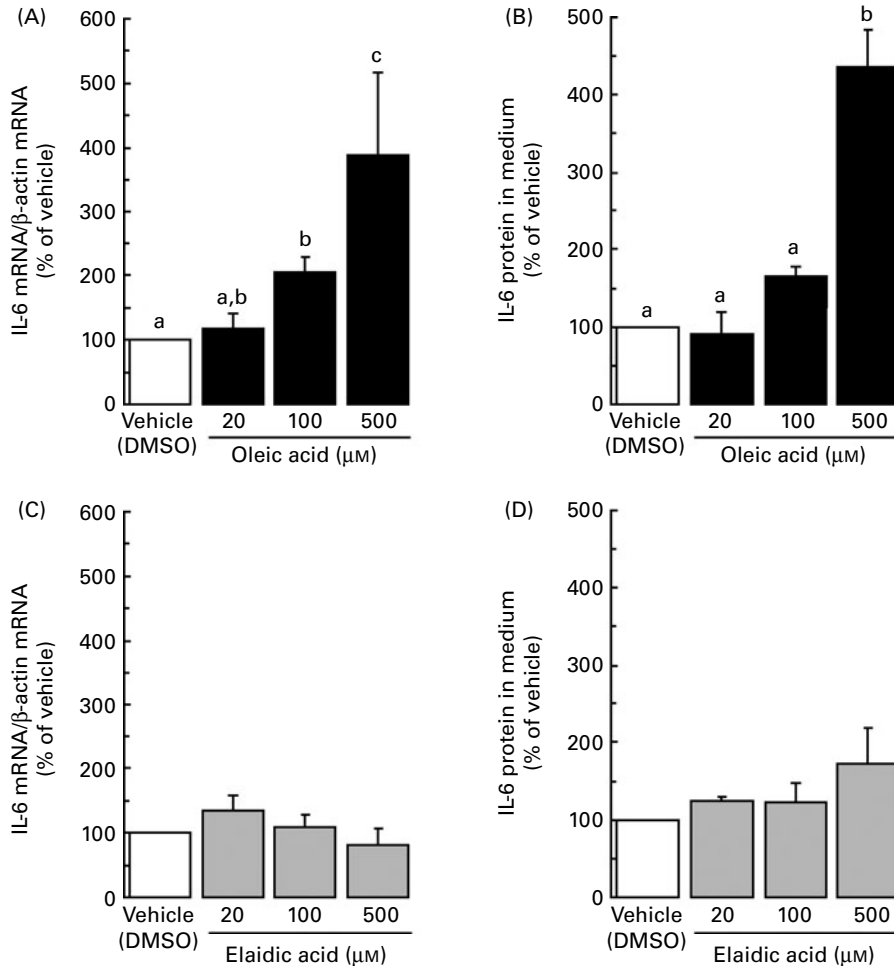
(product no. O1008) and EA (product no. E4637) were from Sigma (St Louis, MO, USA), unless otherwise indicated. The purity of the fatty acids was at least 99%. 3T3-L1 mouse pre-adipocytes (ATTC CL-173™; LGC Dese-laers SL, Barcelona, Spain) were grown to confluence in basal medium – Dulbecco's modified Eagle's medium with penicillin (50 U/ml), streptomycin (50 µg/ml) and 4 mM-L-glutamine – supplemented with 10% newborn calf serum (Linus, Madrid, Spain) under 8% CO<sub>2</sub> and 92% air at 37°C in six- or twelve-well culture plates. At 2 d after the cells reached confluence (referred to as day 0), they were induced to differentiate in basal medium containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), dexamethasone (1 µM), methylisobutylxanthine (0.5 mM) and insulin (1 µg/ml) for 48 h, followed by 48 h in basal medium containing 10% FBS and insulin (1 µg/ml). The cells were subsequently refed every other day with fresh basal medium supplemented with 10% FBS (without insulin). Adipogenesis was monitored by morphological examination of the cells for lipid accumulation using phase contrast microscopy. The cells were treated with fatty acids along with fresh medium on days 8–10 of culture, when more than 90% had acquired the adipose phenotype. For treatment, stock solutions of fatty acids in dimethyl sulphoxide as the vehicle were prepared at 500× the desired final working concentration and diluted 1:500 with basal medium containing 10% FBS. This mixture was pre-warmed for 45 min at 37°C with vortex every 15 min before being added to the differentiated cells<sup>(23)</sup>. Control cells received the same medium incubated with the corresponding volume of the vehicle (0.2% dimethyl sulphoxide). Experimental parameters were measured following 24 h treatment with the fatty acids or the vehicle.

C2C12 mouse myoblasts (ATTC CRL-1772™; LGC Promo-chem SL, Barcelona, Spain) were cultured at 37°C with

**Table 1.** Primers used in the quantitative PCR

Genes	NCBI no.	Primers	Amplicon (bp)
IL-6	NM_031168.1	F: 5'-TGGGAAATCGTGGAATGAG-3' R: 5'-GAAGGACTCTGGCTTTGTCTT-3'	250
IL-15	NM_008357.1	F: 5'-TGTCTTCATTTTGGGCTGTG-3' R: 5'-TGCAACTGGGATGAAAGTCA-3'	155
TNF-α	NM_013693.2	F: 5'-CGTCGTAGCAAACCACCAA-3' R: 5'-GAGAACCCTGGGAGTAGACAAGG-3'	147
Resistin	NM_022984.3	F: 5'-TTCCTTTTCTTCCCTTGCCCTG-3' R: 5'-CTTTTTCTTCACGAATGTCCC-3'	247
Adiponectin	NM_009605.4	F: 5'-GCTCAGGATGCTACTGTTG-3' R: 5'-TCTCACCCCTTAGGACCAAG-3'	256
Retinol-binding protein 4	NM_001159487.1	F: 5'-ACTGGGGTGTAGCCTCCTTT-3' R: 5'-GGTGTCTGTAGTCCGTGTCG-3'	71
GLUT1	NM_011400.3	F: 5'-GCCCCGCTTCTGCTCATC-3' R: 5'-CCCGCATCATCTGCCGACCC-3'	132
GLUT4	NM_009204.2	F: 5'-GGCATGCGTTTCCAGTATGT-3' R: 5'-TGCCCTCAGTCATTCTCATC-3'	234
β-Actin	NM_007393.3	F: 5'-TACAGCTTACCACCACAGC-3' R: 5'-TCTCCAGGGAGGAAGAGGAT-3'	120

F, forward; R, reverse.



**Fig. 1.** Effects of 24 h exposure to varying doses of oleic acid or elaidic acid on IL-6 gene expression and secreted levels in C2C12 myotubes. Gene expression of IL-6 ((A) and (B)) was measured by real-time quantitative PCR and normalised to  $\beta$ -actin. IL-6 protein in the medium ((C) and (D)) was measured by ELISA. Values are means of at least three experiments ( $n$  3–7) each run in triplicate, with standard errors represented by vertical bars, and are expressed relative to the mean value in the vehicle dimethyl sulphoxide (DMSO)-treated control cells, which was set at 100%. The absolute mean value of IL-6 in the medium of the vehicle-treated cells was 429 (SE 62) pg/mg of cell protein. Data were analysed by one-way ANOVA followed by the least significant difference *post hoc* test. <sup>a,b,c</sup> Mean values with unlike letters were significantly different ( $P < 0.05$ ).

humidified 95% air and 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium supplemented with 10% FBS, penicillin (50 U/ml), streptomycin (50  $\mu$ g/ml) and 3 mM-L-glutamine (growth medium) in twelve-well culture plates. To induce differentiation, C2C12 myoblasts were allowed to reach 80% confluence in growth medium and then changed to Dulbecco's modified Eagle's medium containing 2% horse serum (differentiation medium). After ten additional days, the cells had differentiated and fused into myotubes, as assessed by microscopic examination. Differentiated myotubes were treated with the fatty acids or the vehicle for 24 h using the same procedure described above for adipocytes, before RNA isolation or the measurement of glucose uptake.

#### RNA isolation and analysis

Total RNA was extracted from 3T3-L1 adipocytes and C2C12 myotubes using Tripure reagent (Roche, Barcelona, Spain).

Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA), and its integrity was confirmed using agarose gel electrophoresis. A<sub>260</sub>:A<sub>280</sub> ratios were between 1.9 and 2 for all samples, indicating good RNA purity. Retrotranscription followed by real-time quantitative PCR was used to measure the mRNA levels of resistin, adiponectin, retinol-binding protein 4, IL-6, IL-15, TNF- $\alpha$ , GLUT1, GLUT4 and  $\beta$ -actin as an internal control. In brief, 0.25  $\mu$ g of total RNA (in a final volume of 5  $\mu$ l) was denatured at 65°C for 10 min and then reverse transcribed to complementary DNA using murine leukaemia virus RT (Applied Biosystems, Madrid, Spain) in the presence of 50 pmol of random primers in an Applied Biosystems 2720 Thermal Cycler. Sense and antisense primers used in the PCR were designed with specific primer analysis software Primer3 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA), and their specificity was analysed by the ENTREZ and BLAST

database utilities (National Center for Biotechnology Information, Bethesda, MD, USA). The primers were produced by Sigma (Madrid, Spain), and their sequences are shown in Table 1. Each PCR was performed from diluted (1:20) complementary DNA template, forward and reverse primers (1  $\mu$ M each), and Power SYBR Green PCR Master Mix (Applied Biosystems, Madrid, Spain) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). In order to verify the purity of the products, a melting curve was produced after each run according to the manufacturer's instructions. The relative expression of each mRNA was calculated according to Pfaffl<sup>(24)</sup>, using  $\beta$ -actin as a reference gene.

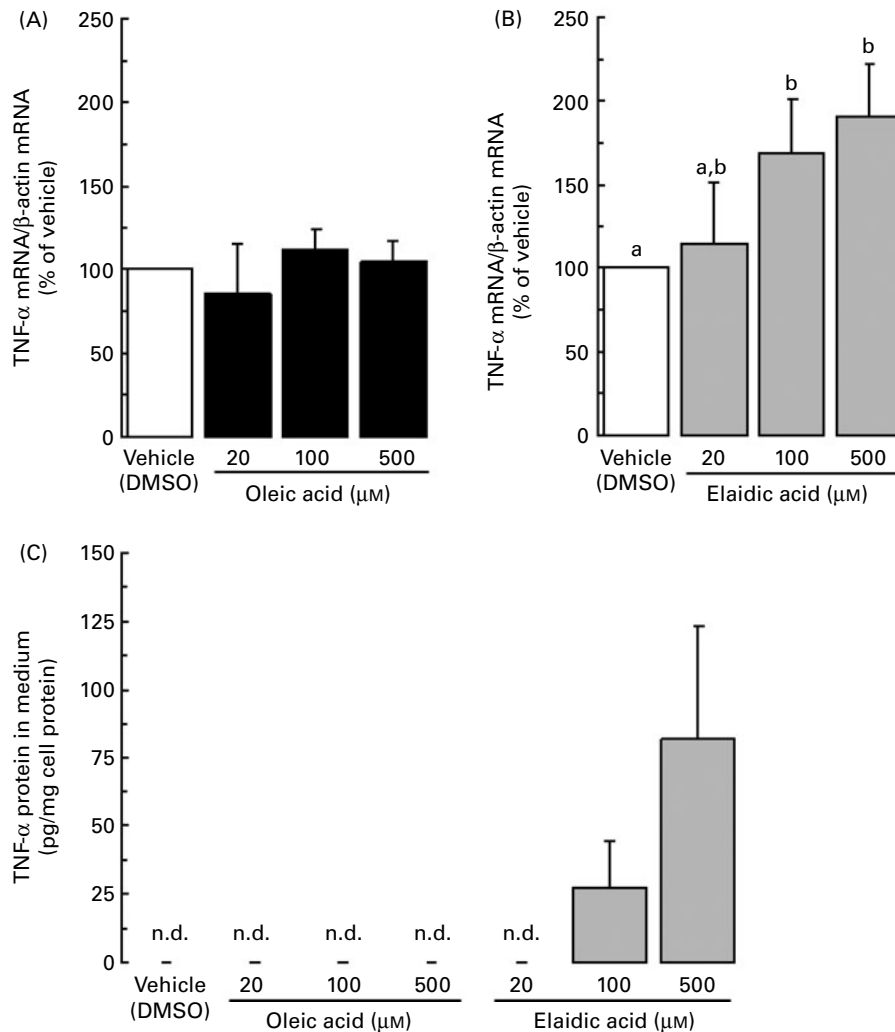
**Quantification of cytokines**

IL-6 and TNF- $\alpha$  concentrations in the cell's conditioned culture medium were measured using ELISA kits

(both from Pierce, Rockford, IL, USA), following the manufacturer's instructions.

**2-Deoxy-D-glucose uptake**

Analysis of 2-deoxy-D-glucose (2-DOG) uptake was performed immediately after completion of the 24 h incubation with fatty acids, essentially as described previously<sup>(25)</sup>. After washing with Krebs–Ringer phosphate buffer (10 mM-KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, containing 136 mM-NaCl, 4.7 mM-KCl, 1.25 mM-CaCl<sub>2</sub>, 1.25 mM-MgSO<sub>4</sub> and 0.05 % bovine serum albumin), cells were incubated with Krebs–Ringer phosphate buffer without glucose in the absence of insulin or in the presence of 100 nM-insulin for 15 min at 37°C. The transport assay was then initiated by the addition of <sup>3</sup>H-2-DOG (3700 Bq/ml in 1  $\mu$ M unlabelled 2-DOG), and the cells were further incubated



**Fig. 2.** Effects of 24 h exposure to varying doses of oleic acid or elaidic acid on TNF- $\alpha$  gene expression and secreted levels in C2C12 myotubes. Gene expression of TNF- $\alpha$  ((A) and (B)) was measured by real-time quantitative PCR and normalised to  $\beta$ -actin, and is expressed relative to the mean value in the vehicle dimethyl sulphoxide (DMSO)-treated control cells, which was set at 100 %. TNF- $\alpha$  protein in the medium (C) was measured by ELISA and is expressed as absolute values (n.d., not detected). Values are means of at least three experiments (*n* 3–7) each run in triplicate, with standard errors represented by vertical bars. Gene expression data were analysed by one-way ANOVA followed by the least significant difference *post hoc* test. <sup>a,b</sup> Mean values with unlike letters were significantly different (*P* < 0.05).

for 10 min with or without 100 nM-insulin at 37°C. Cells were then washed three times with ice-cold PBS and lysed with 0.1 M-NaOH. Radioactivity in cell lysates was counted in Hi-safe 3 scintillant (Perkin Elmer, Shelton, CT, USA) using a Beckman Coulter LS 6500 multi-purpose liquid scintillation counter (Beckman Coulter, Brea, CA, USA). Counting efficiency was about 47%. Protein concentration in cell lysates was evaluated via the bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA).

### Statistical analysis

Data are expressed as means with their standard errors of at least three individual experiments, each run in triplicate or quadruplicate. Differences between treatments were assessed by one-way ANOVA, followed by a least significant differences *post hoc* comparison. Student's *t* test was used to assess the effect of insulin on glucose uptake. The threshold of significance was set at  $P < 0.05$ .

## Results

### Distinct effects of oleic acid and elaidic acid on myokine expression and insulin-stimulated 2-deoxy-D-glucose uptake in C2C12 myotubes

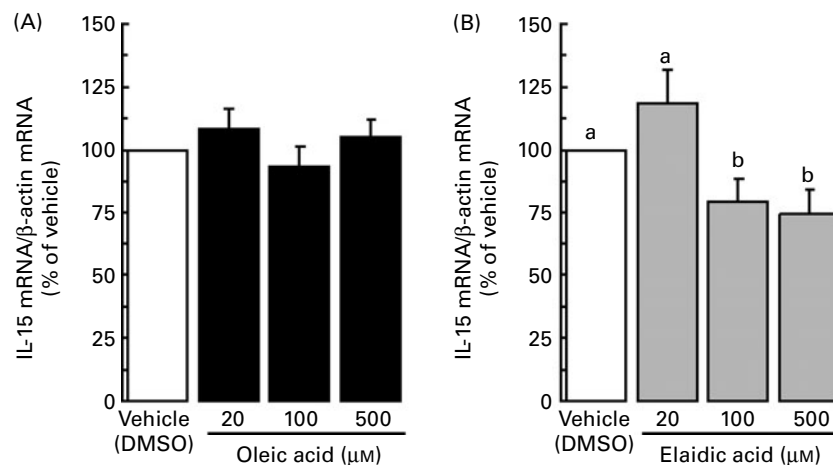
Differentiated C2C12 myotubes were pretreated for 24 h with 20–500  $\mu\text{M}$ -OA, 20–500  $\mu\text{M}$ -EA or the vehicle (0.2% dimethyl sulphoxide), and the changes elicited by fatty acid treatment on IL-6, TNF- $\alpha$  and IL-15 expression were analysed. Treatment with OA caused a dose-dependent increase in cellular IL-6 mRNA levels (Fig. 1(A)) and in IL-6 protein levels in the cell's conditioned culture medium (Fig. 1(B)), whereas treatment with EA had no effect on IL-6 expression either at the mRNA or at the secreted protein level (Fig. 1(C) and (D)). TNF- $\alpha$  mRNA levels were unaffected by OA treatment (Fig. 2(A)) and

were dose dependently increased following treatment with EA by 69% at the 100  $\mu\text{M}$  dose and by 90% at the 500  $\mu\text{M}$  dose (Fig. 2(B)). In good concordance with the mRNA results, TNF- $\alpha$  protein was undetectable in the conditioned culture medium of the vehicle- and OA-treated cells, yet it could be consistently detected, though at very low levels, in the medium of cells treated with the highest EA doses (Fig. 2(C)). IL-15 mRNA levels were unaffected by OA treatment (Fig. 3(A)) and were significantly reduced by about 25% following treatment with 100 or 500  $\mu\text{M}$ -EA (Fig. 3(B)).

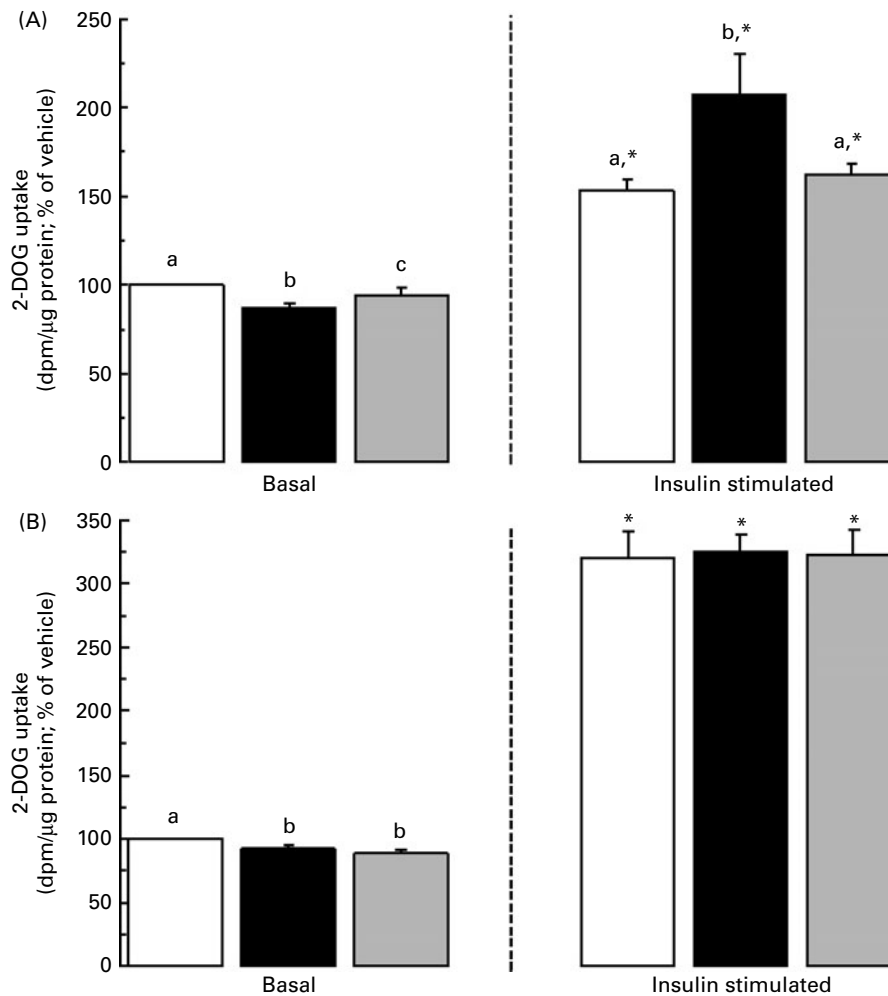
Basal and insulin-stimulated 2-DOG uptake was measured in differentiated C2C12 myotubes following 24 h exposure to 100  $\mu\text{M}$ -OA, 100  $\mu\text{M}$ -EA or the vehicle (Fig. 4(A)). Basal 2-DOG uptake was slightly but significantly lower in the cells that had been treated with either fatty acid compared with the vehicle-treated cells. Stimulation of the cells with 100 nM-insulin produced, as expected, a significant increase in 2-DOG uptake. Insulin-stimulated 2-DOG uptake was approximately 35% higher ( $P < 0.05$ ) in the OA-treated cells compared with the vehicle- or EA-treated cells. Reduced basal 2-DOG uptake in fatty acid-treated myotubes was not related to reduced GLUT1 mRNA expression levels, which did not show differences between treatments (data not shown). Likewise, increased insulin-stimulated 2-DOG uptake in the OA-treated cells was not related to the differences in GLUT4 mRNA levels in these cells compared with the vehicle- or EA-treated cells (data not shown).

### Distinct effects of oleic acid and elaidic acid on adipokine expression in 3T3-L1 adipocytes

Adipokine mRNA levels and 2-DOG uptake were measured in differentiated 3T3-L1 adipocytes following 24 h exposure to 100  $\mu\text{M}$ -OA, 100  $\mu\text{M}$ -EA or the vehicle



**Fig. 3.** Effects of 24 h exposure to varying doses of (A) oleic acid or (B) elaidic acid on IL-15 gene expression in C2C12 myotubes. Values are means of at least three experiments ( $n$  3–7) each run in triplicate, with standard errors represented by vertical bars, and are expressed relative to the mean value in the vehicle dimethyl sulphoxide (DMSO)-treated control cells, which was set at 100%. Gene expression of IL-15 was measured by real-time quantitative PCR and normalised to  $\beta$ -actin. Data were analysed by one-way ANOVA followed by the least significant difference *post hoc* test. <sup>a,b</sup>Mean values with unlike letters were significantly different ( $P < 0.05$ ).



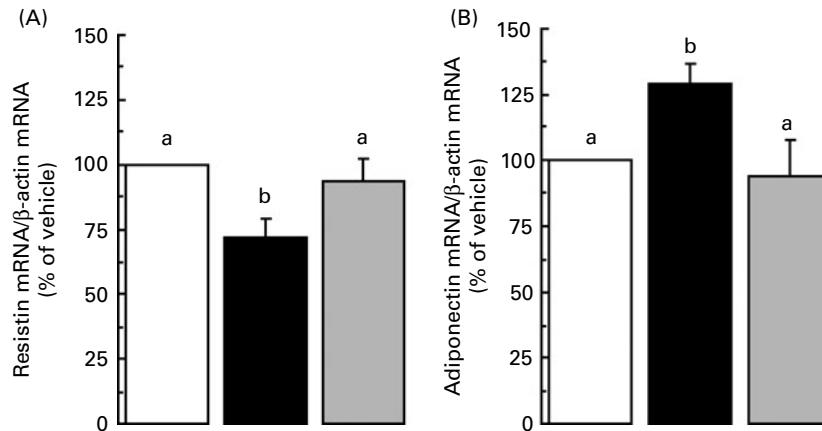
**Fig. 4.** Effects of 24 h exposure to 100  $\mu\text{M}$ -oleic or -elaidic acid on basal and insulin-stimulated 2-deoxy-D-glucose (2-DOG) uptake in (A) C2C12 myotubes and (B) 3T3-L1 adipocytes. 2-DOG uptake was measured in the absence of insulin (basal) and in the presence of 100 nM-insulin as described in the Experimental methods. Values are means of at least three experiments ( $n$  3–8) each run in quadruplicate, with standard errors represented by vertical bars, and are expressed relative to the mean value in the vehicle dimethyl sulphoxide (DMSO)-treated control cells, which was set at 100%. One-way ANOVA followed by the least significant difference *post hoc* test was used to analyse the significance of differences among treatments under the basal and insulin-stimulated conditions;  $a, b, c$  for each condition, mean values with unlike letters were statistically different ( $P < 0.05$ ). \* Statistically significant difference ( $P < 0.05$ ) v. the basal condition assessed by Student's *t* test.  $\square$ , Vehicle (DMSO);  $\blacksquare$ , 100  $\mu\text{M}$ -oleic acid;  $\square$ , 100  $\mu\text{M}$ -elaidic acid; dpm, disintegrations per min.

(0.2% dimethyl sulphoxide), and the changes elicited by fatty acid treatment relative to the vehicle were analysed. Treatment with EA did not affect resistin or adiponectin mRNA levels, whereas treatment with OA resulted in a significant 28% decrease in resistin mRNA levels and a significant 25% increase in adiponectin mRNA levels (Fig. 5). Similar results were obtained in a separate experiment in which the fatty acids were assayed at 500  $\mu\text{M}$  concentration (data not shown). The mRNA levels of the other cytokines/adipokines assayed, namely IL-6, IL-15, TNF- $\alpha$  and retinol-binding protein 4, were very low in 3T3-L1 adipocytes, both under the control condition and after 24 h treatment with OA or EA up to 500  $\mu\text{M}$  (crossing points  $\geq 30$  in the real-time quantitative PCR). Low basal levels of expression of retinol-binding protein 4 and IL-15 mRNA in 3T3-L1 adipocytes are in agreement with previous reports<sup>(26,27)</sup>. No differential effects of OA and EA on 2-DOG uptake in 3T3-L1 adipocytes were found:

pretreatment with either fatty acid similarly slightly lowered basal 2-DOG uptake relative to the vehicle, and insulin-stimulated 2-DOG uptake was equal in adipocytes pre-exposed to OA, EA or the vehicle (Fig. 4(B)).

## Discussion

Skeletal muscle plays a major role in insulin-stimulated glucose disposal<sup>(28)</sup>, and is nowadays recognised as a secretory organ that produces and releases bioactive proteins exerting autocrine, paracrine and endocrine effects, including local effects on muscle glucose uptake<sup>(29)</sup>. Among these is IL-6, a cytokine produced by many cell types and by muscle fibres in response to contraction<sup>(29,30)</sup>. IL-6 enhances glucose uptake by human and rat skeletal muscle cells<sup>(31–33)</sup>, and secretion of IL-6 is reduced in human skeletal muscle cells that are made insulin-resistant by incubation with



**Fig. 5.** Effects of 24 h exposure to 100  $\mu$ M-oleic or -elaidic acid on the gene expression of (A) resistin and (B) adiponectin in 3T3-L1 adipocytes. Values are means of four experiments ( $n$  4) each run in triplicate, with standard errors represented by vertical bars, and are expressed relative to the mean value in the vehicle dimethyl sulphoxide (DMSO)-treated control cells, which was set at 100%. Gene expression was measured by real-time quantitative PCR and normalised to  $\beta$ -actin. Data were analysed by one-way ANOVA followed by the least significant difference *post hoc* test. <sup>a,b</sup> Mean values with unlike letters were significantly different ( $P < 0.05$ ).  $\square$ , Vehicle (DMSO);  $\blacksquare$ , 100  $\mu$ M-oleic acid;  $\blacksquare$ , 100  $\mu$ M-elaidic acid.

adipocyte-conditioned medium<sup>(34)</sup>. These and other findings, including reports of central and peripheral anti-obesity effects of IL-6, have challenged the classical view of IL-6 as a pro-inflammatory cytokine associated with obesity and reduced insulin sensitivity<sup>(29,30)</sup>. Our finding that incubation with OA increased both IL-6 expression and insulin-stimulated 2-DOG uptake in C2C12 myotubes supports this novel scenario for muscle-derived IL-6. The present results are in concordance with reported stimulatory effects of IL-6 on skeletal muscle glucose uptake<sup>(31–33)</sup> and with reported beneficial effects of OA on insulin sensitivity, systemically and at the skeletal muscle level<sup>(4,5,35)</sup>. In particular, OA has been shown to protect against palmitate-induced insulin resistance at the level of glucose uptake in rat L6 myotubes<sup>(35)</sup>. In that study<sup>(35)</sup>, however, OA alone did not increase insulin-stimulated glucose uptake as in ours; the reasons for this difference remain unclear and may relate to differences in the cell model and treatment conditions used. Importantly, we found that the stimulatory effects of OA on insulin-stimulated 2-DOG uptake and IL-6 expression at both the mRNA and secreted protein levels in skeletal muscle cells were not reproduced by its *trans*-isomer, EA.

Treatment with either OA or EA caused in our hands a small, but consistent, reduction in cellular basal 2-DOG glucose. The present results indicate that the observed effects of fatty acids on 2-DOG uptake in muscle cells were unrelated to changes in GLUT1 or GLUT4 mRNA levels. Muscle has a high potential to adjust fuel selection depending on energy supply and demand. Fatty acids have been shown to induce the expression in muscle cells (including C2C12 myotubes) of key genes involved in their catabolism, which may facilitate the transition from a more glycolytic to a more lipid-oxidative metabolism during episodes of heavy influx of fatty acids to skeletal muscle, such as in fasting<sup>(36)</sup>. It is possible, therefore, that reduced basal glucose uptake may,

by default, result as a consequence of increased cellular fatty acid catabolism. In any case, the significance and mechanistic basis of fatty acid effects on glucose uptake observed in the present study requires further investigation.

IL-15 is another myokine possibly involved in the control of muscle glucose disposal. IL-15 is highly expressed in skeletal muscle compared with other tissues and plays important regulatory roles in skeletal muscle itself, where it promotes protein synthesis, fatty acid oxidation, and glucose uptake and utilisation<sup>(37,38)</sup>. In addition to anti-diabetogenic effects, anti-adiposity effects have been proposed for muscle-derived IL-15 acting on multiple target tissues including adipose tissue<sup>(38,39)</sup>. We report here, for the first time to our knowledge, a down-regulatory effect of EA on IL-15 gene expression in skeletal muscle cells, which could have implications for the effects of TFA promoting insulin resistance and obesity evidenced in previous studies<sup>(12,13,15–17,19–21)</sup>.

Adipose tissue plays a relatively modest role in insulin-stimulated glucose disposal compared with skeletal muscle, yet it contributes to the control of systemic insulin sensitivity through the secretion of NEFA and adipokines that have an impact on skeletal muscle and liver metabolism, among other mechanisms. Two important adipokines in this context are resistin and adiponectin, which exert opposite effects as an insulin resistance factor<sup>(40,41)</sup> and an insulin-sensitising factor<sup>(42)</sup>, respectively. Resistin and adiponectin might also oppositely relate to inflammation, as there is evidence of pro-inflammatory properties of resistin<sup>(41)</sup> and anti-inflammatory properties of adiponectin especially with regard to atherosclerosis<sup>(43)</sup>. In our hands, exposure to OA significantly reduced gene expression of resistin and increased that of adiponectin in mature 3T3-L1 adipocytes, and these putatively beneficial effects were not reproduced by EA, which did not affect adiponectin or resistin gene

expression. We also investigated the effects on retinol-binding protein 4 – which, similar to resistin, might function as an insulin resistance factor secreted by adipocytes<sup>(44)</sup> – and found no effect of OA or EA treatment on its (very low) gene expression in 3T3-L1 adipocytes. Repression of adipocyte resistin expression by OA has been previously reported<sup>(45)</sup>, and a stimulatory effect of OA on adiponectin expression might be in line with a recent report showing a positive association between serum adiponectin levels and OA content in white adipose tissue in rats<sup>(46)</sup>. In a previous study in 3T3-L1 adipocytes, however, 48 h incubation with 250  $\mu$ M-OA had no effect on adiponectin gene expression<sup>(47)</sup>. Studies examining the effects of TFA on adipokine expression in adipocyte cell models are lacking, to our knowledge. There are, however, reports indicating that, in rats, consumption of TFA-enriched diets is associated with increased resistin expression<sup>(48,49)</sup> and reduced adiponectin expression in white adipose tissue<sup>(49,50)</sup>. Additionally, also in rats, a negative correlation between adipose adiponectin gene expression and TFA levels in the serum has been described<sup>(46)</sup>. Taken together, these *in vivo* studies and our cell studies suggest that the effects of TFA-enriched diets on adipokine expression observed *in vivo* are likely to be indirect.

TNF- $\alpha$  is a cytokine linked to inflammation and obesity-associated insulin resistance<sup>(51)</sup>. TFA intake has been reported to be associated with increased TNF system activity in observational studies in humans<sup>(10)</sup> and with increased TNF- $\alpha$  expression in white adipose tissue in experimental studies in rats<sup>(49,50)</sup>. In our hands, gene expression of TNF- $\alpha$  was negligible in 3T3-L1 adipocytes yet readily detectable in C2C12 myotubes, in which both TNF- $\alpha$  mRNA levels and secreted TNF- $\alpha$  protein increased following exposure to EA. TNF- $\alpha$  activity favours insulin resistance and protein wasting in skeletal muscle<sup>(52)</sup>, effects that are the opposite to those exerted in muscle by IL-15<sup>(38)</sup>, and, remarkably, we found here that EA oppositely affected gene expression of these two cytokines in muscle cells.

In summary, the present study revealed the distinct, direct effects of OA and EA on the expression of target myokines and adipokines. OA, but not EA, dose dependently induced IL-6 expression in skeletal muscle cells, and repressed resistin and induced adiponectin gene expression in cultured adipocytes. EA, but not OA, repressed IL-15 gene expression and induced TNF- $\alpha$  expression in cultured skeletal muscle cells. Overall, these findings suggest novel mechanisms by which *cis*- and *trans*-monounsaturated fat may differentially affect systemic functions.

### Acknowledgements

The present study was supported by grants from the Spanish Government (AGL2009-11277/ALI to A. P.) and

the European Union (BIOCLAIMS project, FP7-244995, Coordinator A. P.). Our laboratory consists of a member of the European Nutrigenomics Organization (NuGO, European Union Contract FOOD-CT-2004-506360). CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of the ISCIII (Spanish Government). All authors contributed to the design and discussion of the study. N. G. and J. A. undertook all the experiments. N. G. drafted the manuscript. J. R., A. P. and M. L. B. supervised the experiments. M. L. B. was in charge of the final writing of the manuscript. The authors declare that there is no conflict of interest.

### References

1. Mensink RP (2005) Metabolic and health effects of isomeric fatty acids. *Curr Opin Lipidol* **16**, 27–30.
2. Stender S, Astrup A & Dyerberg J (2008) Ruminant and industrially produced *trans*-fatty acids: health aspects. *Food Nutr Res* **52** (epublication 12 March 2008).
3. Covas MI (2007) Olive oil and the cardiovascular system. *Pharmacol Res* **55**, 175–186.
4. Schroder H (2007) Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. *J Nutr Biochem* **18**, 149–160.
5. Riserus U, Willett WC & Hu FB (2009) Dietary fats and prevention of type 2 diabetes. *Prog Lipid Res* **48**, 44–51.
6. Mozaffarian D, Aro A & Willett WC (2009) Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* **63**, Suppl. 2, S5–S21.
7. Teegala SM, Willett WC & Mozaffarian D (2009) Consumption and health effects of *trans* fatty acids: a review. *J AOAC Int* **92**, 1250–1257.
8. Bhattacharya A, Banu J, Rahman M, *et al.* (2006) Biological effects of conjugated linoleic acids in health and disease. *J Nutr Biochem* **17**, 789–810.
9. Field CJ, Blewett HH, Proctor S, *et al.* (2009) Human health benefits of vaccenic acid. *Appl Physiol Nutr Metab* **34**, 979–991.
10. Mozaffarian D (2006) *Trans* fatty acids – effects on systemic inflammation and endothelial function. *Atheroscler Suppl* **7**, 29–32.
11. Lovejoy JC, Smith SR, Champagne CM, *et al.* (2002) Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or *trans* (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care* **25**, 1283–1288.
12. Odegaard AO & Pereira MA (2006) *Trans* fatty acids, insulin resistance, and type 2 diabetes. *Nutr Rev* **64**, 364–372.
13. Riserus U (2006) *Trans* fatty acids and insulin resistance. *Atheroscler Suppl* **7**, 37–39.
14. Tardy AL, Lambert-Porcheron S, Malpuech-Brugere C, *et al.* (2009) Dairy and industrial sources of *trans* fat do not impair peripheral insulin sensitivity in overweight women. *Am J Clin Nutr* **90**, 88–94.
15. Natarajan S & Ibrahim A (2005) Dietary *trans* fatty acids alter diaphragm phospholipid fatty acid composition, triacylglycerol content and glucose transport in rats. *Br J Nutr* **93**, 829–833.
16. Ibrahim A, Natrajan S & Ghafoorunissa R (2005) Dietary *trans*-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats. *Metabolism* **54**, 240–246.



17. Dorfman SE, Laurent D, Gounarides JS, *et al.* (2009) Metabolic implications of dietary *trans*-fatty acids. *Obesity (Silver Spring)* **17**, 1200–1207.
18. Tardy AL, Giraudet C, Rousset P, *et al.* (2008) Effects of *trans* MUFA from dairy and industrial sources on muscle mitochondrial function and insulin sensitivity. *J Lipid Res* **49**, 1445–1455.
19. Kavanagh K, Jones KL, Sawyer J, *et al.* (2007) *Trans* fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. *Obesity (Silver Spring)* **15**, 1675–1684.
20. Koh-Banerjee P, Chu NF, Spiegelman D, *et al.* (2003) Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. *Am J Clin Nutr* **78**, 719–727.
21. Field AE, Willett WC, Lissner L, *et al.* (2007) Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity (Silver Spring)* **15**, 967–976.
22. Ritchie SA & Connell JM (2007) The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutr Metab Cardiovasc Dis* **17**, 319–326.
23. Amri EZ, Teboul L, Vannier C, *et al.* (1996) Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. *Biochem J* **314** (Pt 2), 541–546.
24. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45.
25. Kumar N & Dey CS (2003) Development of insulin resistance and reversal by thiazolidinediones in C2C12 skeletal muscle cells. *Biochem Pharmacol* **65**, 249–257.
26. Mercader J, Granados N, Bonet ML, *et al.* (2008) All-*trans* retinoic acid decreases murine adipose retinol binding protein 4 production. *Cell Physiol Biochem* **22**, 363–372.
27. Quinn LS, Strait-Bodey L, Anderson BG, *et al.* (2005) Interleukin-15 stimulates adiponectin secretion by 3T3-L1 adipocytes: evidence for a skeletal muscle-to-fat signaling pathway. *Cell Biol Int* **29**, 449–457.
28. Parish R & Petersen KF (2005) Mitochondrial dysfunction and type 2 diabetes. *Curr Diab Rep* **5**, 177–183.
29. Pedersen BK & Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* **88**, 1379–1406.
30. Hoene M & Weigert C (2008) The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. *Obes Rev* **9**, 20–29.
31. Carey AL, Steinberg GR, Macaulay SL, *et al.* (2006) Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation *in vitro* via AMP-activated protein kinase. *Diabetes* **55**, 2688–2697.
32. Al-Khalili L, Bouzakri K, Glund S, *et al.* (2006) Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. *Mol Endocrinol* **20**, 3364–3375.
33. Glund S, Deshmukh A, Long YC, *et al.* (2007) Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. *Diabetes* **56**, 1630–1637.
34. Sell H, Eckardt K, Taube A, *et al.* (2008) Skeletal muscle insulin resistance induced by adipocyte-conditioned medium: underlying mechanisms and reversibility. *Am J Physiol Endocrinol Metab* **294**, E1070–E1077.
35. Gao D, Griffiths HR & Bailey CJ (2009) Oleate protects against palmitate-induced insulin resistance in L6 myotubes. *Br J Nutr* **102**, 1557–1563.
36. Rodriguez AM, Sanchez J, Tobaruela A, *et al.* (2010) Time-course effects of increased fatty acid supply on the expression of genes involved in lipid/glucose metabolism in muscle cells. *Cell Physiol Biochem* **25**, 337–346.
37. Busquets S, Figueras M, Almendro V, *et al.* (2006) Interleukin-15 increases glucose uptake in skeletal muscle. An antidiabetogenic effect of the cytokine. *Biochim Biophys Acta* **1760**, 1613–1617.
38. Argiles JM, Lopez-Soriano FJ & Busquets S (2009) Therapeutic potential of interleukin-15: a myokine involved in muscle wasting and adiposity. *Drug Discov Today* **14**, 208–213.
39. Quinn LS, Anderson BG, Strait-Bodey L, *et al.* (2009) Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. *Am J Physiol Endocrinol Metab* **296**, E191–E202.
40. Lazar MA (2007) Resistin- and obesity-associated metabolic diseases. *Horm Metab Res* **39**, 710–716.
41. Barnes KM & Miner JL (2009) Role of resistin in insulin sensitivity in rodents and humans. *Curr Protein Pept Sci* **10**, 96–107.
42. Kadowaki T, Yamauchi T, Kubota N, *et al.* (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* **116**, 1784–1792.
43. Stofkova A (2009) Leptin and adiponectin: from energy and metabolic dysbalance to inflammation and autoimmunity. *Endocr Regul* **43**, 157–168.
44. Yang Q, Graham TE, Mody N, *et al.* (2005) Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* **436**, 356–362.
45. Rea R & Donnelly R (2006) Effects of metformin and oleic acid on adipocyte expression of resistin. *Diabetes Obes Metab* **8**, 105–109.
46. Perez de Heredia F, Sanchez J, Priego T, *et al.* (2009) Adiponectin is associated with serum and adipose tissue fatty acid composition in rats. *J Endocrinol Invest* **32**, 659–665.
47. Bueno AA, Oyama LM, de Oliveira C, *et al.* (2008) Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL/6J mice adipose tissue. *Pflugers Arch* **455**, 701–709.
48. Saravanan N, Haseeb A, Ehtesham NZ, *et al.* (2005) Differential effects of dietary saturated and *trans*-fatty acids on expression of genes associated with insulin sensitivity in rat adipose tissue. *Eur J Endocrinol* **153**, 159–165.
49. Duque-Guimaraes DE, de Castro J, Martinez-Botas J, *et al.* (2009) Early and prolonged intake of partially hydrogenated fat alters the expression of genes in rat adipose tissue. *Nutrition* **25**, 782–789.
50. Pisani LP, Oyama LM, Bueno AA, *et al.* (2008) Hydrogenated fat intake during pregnancy and lactation modifies serum lipid profile and adipokine mRNA in 21-day-old rats. *Nutrition* **24**, 255–261.
51. Ryden M & Arner P (2007) Tumour necrosis factor- $\alpha$  in human adipose tissue – from signalling mechanisms to clinical implications. *J Intern Med* **262**, 431–438.
52. Argiles JM, Alvarez B, Carbo N, *et al.* (2000) The divergent effects of tumour necrosis factor- $\alpha$  on skeletal muscle: implications in wasting. *Eur Cytokine Netw* **11**, 552–559.