Time Course of Changes in the Expression of Insulin Sensitivity-Related Genes after an Acute Load of Virgin Olive Oil

Valentini Konstantinidou,1 Olha Khymenets,2 Maria-Isabel Covas,1 Rafael de la Torre,2,3 Daniel Muñoz-Aguayo,1 Roger Anglada,3 Magi Farré,2,4 and Montserrat Fito1

Abstract

Our aim was to examine whether an acute fat load could induce changes in the expression of insulin sensitivity-related genes in human peripheral blood mononuclear cells. Selection of candidate genes was based on previous studies with sustained virgin olive oil (VOO) consumption and biological plausibility in relation to insulin sensitivity. Eleven healthy volunteers ingested raw VOO (50 mL). Blood samples were collected at 0, 1 and 6 h. Plasma glucose, insulin and hydroxytyrosol increased at 1 h and decreased at 6 h. Lipid oxidative damage increased at 6 h (p < 0.05). Gene expression changes were characterized based on quantification of the samples relative to a reference sample [i.e., relative quantification (RQ) method]. A 1 h downregulation was observed in O-linked-N-acetylglucosamine transferase (OGT, RQ: 0.62/C6 0.32) and arachidonate-5-lipoxygenase-activating protein (ALOX5AP, RQ: 0.64/C6 0.31) genes (p < 0.005). OGT was upregulated at 6 h (RQ: 1.88/C6 0.28, p < 0.05). CD36 (thrombospondin receptor) was upregulated at 1 h (RQ: 1.6 ± 0.8, p < 0.05) returning to the basal values at 6 h. Lipoic acid synthetase (LIAS), peroxisome proliferator-activated receptor binding protein (PPARBP), a disintegrin and metallopeptidase domain 17 (ADAM17), and adrenergic beta-2-receptor (ADRB2) genes were upregulated at 6 h (range for the mean RQ: 1.33–1.56) following an increasing linear trend (p < 0.05) from baseline to 6 h. ALOX5AP and OGT genes inversely correlated with insulin and glucose levels at 1 h. ADAM17 and ADRB2 inversely correlated with oxLDL at 6 h (p < 0.05). Taken together, these observations may inform the future clinical nutrigenomics study designs and indicate that a single dose of VOO can elicit quantifiable and rapid changes in gene expression in targets that are mechanistically relevant for insulin sensitivity and the metabolic syndrome.

Introduction

Postprandial hyperlipidemia, hyperglycaemia, oxidative stress, and insulin resistance may occur after meals with a high fat content (Roche and Gibney, 2000). Postprandial lipidemia has been recognized as a risk factor for atherosclerosis development, as it is associated with oxidative changes (Regnstrom et al., 1992). The impaired ability to eliminate lipids in the postprandial state is an atherogenic trait associated with insulin resistance. Excessive postprandial hyperglycaemia is directly toxic to the endothelium, increasing protein glycation, generating oxidative stress, and causing impaired endothelial function (Ceriello, 1999; 2000; Williams et al., 1998). Hyperinsulinemia itself may also be pathogenic (Pyorala et al., 1998; Stout, 1996). Insulin resistance and compensatory hyperinsulinemia are involved in the development of dyslipidemia, hypertension, impaired fibrinolysis, and other abnormalities that collectively contribute to an increased risk of coronary heart disease (CHD) (Steiner and Lewis, 1996; Zavaroni et al., 1999).

In the present study, seven insulin sensitivity-related genes were selected to analyze their expression changes after an acute oral ingestion of 50-mL raw virgin olive oil (VOO). The selection of the genes was performed on the basis of the
atherosclerosis-related responsive genes observed in peripheral blood mononuclear cells (PBMCs) of healthy volunteers after long-term (3 weeks) consumption of 25-mL VOO per day (Khymenets et al., 2009). In that work, 23 responsive genes were identified based on the microarray results and their further screening. Careful and detailed bibliographic research (PubMed database http://pubmed.gov) revealed that seven of them (ADAM17, a disintegrin and metalloproteinase domain 17; ADRB2, adrenergic beta-2-receptor; ALOX5AP, arachidonate 5-lipoxygenase-activating protein; CD36, CD36 (thrombospondin receptor); LIAS, lipoic acid synthetase; OGT, O-linked N-acetylglucosamine (O-GlcNac) transferase and PPARGP, peroxisome proliferator-activated receptor binding protein) were related to the insulin sensitivity mechanisms (Fujimura et al., 2006; Handberg et al., 2009; Kaaman et al., 2006; Pershadsingh, 2007; Philipson, 2002; Togashi et al., 2002; Whelan et al., 2008). Notwithstanding these results from longer term administration of VOO, it is also of interest to evaluate whether and to what extent these genes respond to acute administration of VOO.

Insulin plays a central role in determining the triglycerides turnover and clearance, via lipoprotein lipase activation, through the synthesis and secretion of very low density lipoproteins (VLDL) (Berge et al., 2005). Insulin secretion can be divided into two different phases, the stimulated (postprandial) state that regulates glucose metabolism when carbohydrate is abundant and must be disposed of, and the basal (postabsorptive) state that prevails during the interprandial phases. Long-term maintenance of serum glucose concentrations is a closely regulated process in mammalian species (Henriksen, 2006). Great variations in insulin sensitivity are common even among young healthy individuals (Pedersen, 1999). Less than one-third of the interindividual variation in insulin sensitivity is explained by known factors such as obesity. Thus, genetic factors along with environmental influences deserve consideration to account for other hitherto neglected contributions that can explain this large variation (Riserus, 2008).

Nutrients can regulate the expressed gene products at transcription, mRNA processing, mRNA stability, translation, and/or posttranslational modification stages (Salati et al., 2004). The ability of an individual to cope with a fatty meal may be a key factor in the development of CHD. Nutrient–gene interactions could be involved in the fat clearance, insulin homeostasis, and insulin sensitivity/resistance changes after fat ingestion. To this end, human nutrigenomics data are scarce. We have previously demonstrated that an oral load of 25 mL of any type of olive oil does not promote postprandial hyperlipidemia and oxidative stress in healthy volunteers (Weinbrenner et al., 2004), whereas doses equal to or greater than 40 mL do (Covas et al., 2006; Fito et al., 2002). The aim of the present study was to examine whether an acute oral ingestion of 50-mL raw VOO results in quantifiable changes in the expression of insulin sensitivity-related genes in human PBMCs, and to discern their postprandial time course.

Materials and Methods

Subjects

Eleven healthy volunteers (six male and five female), aged 22 to 44, were recruited. The institutional ethics committee (CEIC-IMAS) approved the protocol and the volunteers signed a specific, written and informed consent. All were healthy on the basis of a physical examination and standard biochemical and haematological tests. Subjects had an average weight of 66.28 ± 12.73 kg, and a body mass index of 23.11 ± 3.06 kg/m². Ten of them participated in our previous study, which examined gene expression changes after sustained (3 weeks) VOO consumption (Khymenets et al., 2009).

Study design and sample collection

Prior to the ingestion of VOO (intervention), subjects followed a 1-week washout period during which sunflower oil was provided as the only source of fat for consumption (raw and cooked). During the first 4 days of this washout period, participants were asked to control their antioxidant intake. During the 3 days prior to the intervention they followed a strict low-phenolic compound diet. At 8 a.m. of the intervention day, at fasting state, 50 mL (44 g) of raw VOO was administered in a single dose with bread (200 g). During the first postprandial 6 h, subjects abstained from food and drinks with the exception of caffeine-free, low-energy drinks, and water. Blood was collected in 8-mL Cell Preparation Tubes BD Vacutainer® CPT™ (Beckton Dickinson, Franklin Lakes, NJ) at baseline (0 h, predose), at 1 h, and at 6 h after VOO ingestion. To ascertain participants’ compliance, the nutritionist verified that they consumed the total amount of VOO administered in the 50-mL containers.

Insulin, hydroxytyrosol, glycaemia, and lipid profile determinations

Insulin levels were measured by an enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden). Glucose and lipid analyses were performed in a PENTRA-400 autoanalyzer (ABX-Horiba Diagnostics, Montpellier, France). Serum glucose, total cholesterol, and triglyceride levels were measured using standard enzymatic automated methods (ABX-Horiba Diagnostics, Montpellier, France). High-density lipoprotein (HDL) cholesterol was directly determined by an accelerator selective detergent method (ABX-Horiba Diagnostics, Montpellier, France). LDL cholesterol was calculated by the Friedewald (Friedewald et al., 1972) formula whenever triglycerides were <300 mg/dL. Plasma lipid peroxides were assessed by the generation of malondialdehyde equivalents, and measured by the thiobarbituric acid reactive substances method. Oxidized low-density lipoprotein (OxLDL) was determined in plasma by a sandwich ELISA procedure using the murine monoclonal antibody mAB-4E6 as capture antibody, and a peroxidase conjugated antibody against oxidized apolipoprotein B bound to the solid phase (ox-LDL, Mercodia AB, Uppsala, Sweden). OxLDL values were adjusted by low-density lipoprotein (LDL) cholesterol. Concentrations of hydroxytyrosol in plasma samples were determined by gas chromatography-mass spectrometry (GC-MS) (Miro-Casas et al., 2003).

RNA extraction

On the basis of our previous experience (Khymenets et al., 2005), the liquid–liquid RNA isolation method from PBMCs using the Ultraspec solution (Biotecx Laboratories, Houston, TX) was utilized. In brief, PBMCs were isolated from peripheral blood using BD Vacutainer® CPT™ cell preparation
Reverse-transcription and real-time qPCR

A total of 100 ng of total RNA in a 20-µL reaction was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems, Foster City, CA) according to manufacturer’s protocols. Quantitative PCR assays (TaqMan® Low-Density Array by Design) were performed using 384-well MicroFluidic cards on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) for seven insulin resistance-related genes: (1) a disintegrin and metalloproteinase domain 17 (also known as tumor necrosis factor-alpha (TNF-OC) converting enzyme, TACE) (ADAM17); (2) adrenergic beta-2-receptor (ADRB2); (3) arachidonate 5-lipoxygenase-activating protein (ALOX5AP); (4) CD36 molecule, thrombospondin receptor, (CD36); (5) lipoic acid synthetase (LIAS); (6) O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT); and (7) peroxisome proliferator-activated receptor binding protein (PPARBP). The context sequences used in the quantitative real-time PCR (qRT-PCR) (Table 1) were presented in Table 1 based on the manufacturer’s indications. The mRNA expressions were normalized by using human glyceraldehyde-3-phosphate dehydrogenase (GADPH) as an endogenous control to correct the differences in the amount of total RNA added to each reaction. The data obtained were analyzed and stored in the SDS 2.1 software. Results from each run were analyzed separately using a software-defined base-line and a threshold of 0.20. The change in gene expression was calculated using the \( 2^{-\Delta\Delta CT} \) relative quantification method (RQ) where data were normalized first to the endogenous reference gene (GADPH) and after to the untreated control (baseline) (Livak and Schmittgen, 2001) according to the manufacturer’s guidelines (Applied Biosystems).

Statistical analysis

Normality of continuous variables was assessed by normal probability plots. Nonparametric data were normalized by log conversion for further statistical analysis. Correlation analyses were performed by Pearson’s correlation test. A general linear model for repeated measurements was used, with multiple paired comparisons corrected by Tukey’s method, in order to assess differences among the time points evaluated. The Student’s t-test for independent samples was performed to assess gender differences. A p-value of ≤0.05 was considered as statistically significant. All statistical analysis was performed by SPSS 12.3 software (SPSS Inc. Chicago, IL) for Windows XP (Microsoft, Redmond, WA).

**Results**

The insulin, glucose, and hydroxytyrosol levels increased at 1 h after the olive oil consumption followed by a decrease at 6 h (\( p < 0.05 \)) (Table 2). An increase in oxidized LDL and lipid peroxides were observed at 6 h postprandial (\( p < 0.05 \)). The quality and purity of the total RNA were ascertained using standard measures (A260/A280 and A260/A230 ≥ 1.8 and 8.5 < RIN < 9.5).

Two different patterns were observed concerning the gene expression profile changes. The first (Fig. 1), followed a quadratic trend that was significant in the case of ALOX5AP and OGT (\( p < 0.01 \), reaching a borderline significance in the case of CD36 (\( p < 0.1 \)). A downregulation at 1 h after olive oil ingestion was observed in OGT and ALOX5AP genes (RQ values, 0.62 ± 0.32 and 0.64 ± 0.31, respectively, \( p < 0.005 \)) followed by an upregulation at 6 h in the case of OGT (RQ: 1.88 ± 0.28). CD36 was upregulated at 1 h (RQ = 1.6 ± 0.8, \( p < 0.05 \)) returning to basal values at 6 h (Fig. 1). The second pattern, followed by LIAS, PPARBP, ADAM17, and ADRB2 showed an upregulation at 6 h (mean RQ range 1.33–1.56, \( p < 0.05 \)) following a linear trend (\( p < 0.05 \)) from baseline to 6 h (Fig. 2). Changes in ADRB2 expression at 6 h postprandial were significantly higher than those at 1 h (\( p < 0.05 \)).

**Table 1. Context Sequences Used in Quantitative Real-Time PCR (qRT-PCR)**

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Assay ID</th>
<th>Context sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM17</td>
<td>ADAM metalloproteinase domain 17 (TACE,Tumour necrosis factor alpha converting enzyme)</td>
<td>Hs00234224_m1</td>
<td>TGTCCAGTGCAAGTGACAGGAACAGT</td>
</tr>
<tr>
<td>ADRB2</td>
<td>Adrenergic beta-2-receptor</td>
<td>Hs00240532_s1</td>
<td>CCACCCACAGGACGATCAACTCTG</td>
</tr>
<tr>
<td>ALOX5AP</td>
<td>Arachidonate 5-lipoxygenase-activating protein</td>
<td>Hs00233463_m1</td>
<td>ACGTCCACCAAGTCACTAGT</td>
</tr>
<tr>
<td>CD36</td>
<td>CD36 molecule (thrombospondin receptor)</td>
<td>Hs00169627_m1</td>
<td>ATGATTATGTCAGATGACGCTT</td>
</tr>
<tr>
<td>LIAS</td>
<td>Lipoic acid synthetase</td>
<td>Hs00398048_m1</td>
<td>CATATAAACAGGCTAATTITTCCT</td>
</tr>
<tr>
<td>OGT</td>
<td>O-linked N-acetylglucosamine (GlcNAc) transferase</td>
<td>Hs00269228_m1</td>
<td>ACTTTGAAAGCTCTGATCAGCC</td>
</tr>
<tr>
<td>PPARBP</td>
<td>PPARg binding protein</td>
<td>Hs00191130_m1</td>
<td>GGGGAGAATCTCCTGAGCTGTCGG</td>
</tr>
</tbody>
</table>
correlations among the expression of the seven selected genes at 1 h and 6 h after olive oil ingestion were obtained, which are shown in Tables 3 and 4. No differences were observed between genders.

Correlation analyses showed that the 1-h insulin increase was inversely related with ALOX5AP expression (r = -0.685, p = 0.020) reaching a borderline significance in the case of OGT (r = -0.570, p = 0.067). The 1 h glucose increase was also inversely correlated not only with ALOX5AP (r = -0.786, p = 0.004), but also with OGT (r = -0.744, p = 0.009) (Supplementary Table 1). (See online supplementary material at www.liebertonline.com). At 6 h postprandial, an inverse correlation was observed between oxidized LDL and ADAM17 and ADRB2 gene expression (r = -0.661, p = 0.027 and r = -0.642, p = 0.033, respectively). ADRB2 up-regulation was also inversely correlated with triglyceride values at 6 h postprandial (r = -0.684, p = 0.027) (Supplementary Table 2). When the relationship between plasma hydroxytyrosol and gene expression changes was assessed, only at 1 h after olive oil ingestion there was an inverse correlation between oxidized LDL and ADRB2 up-regulation.

### Table 2. Insulin, Glucose, Lipid Profile, and Hydroxytyrosol Values at Baseline, 1 Hour and 6 Hours after Olive Oil Ingestion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (0 hours)</th>
<th>1 hour</th>
<th>6 hours</th>
<th>p value for quadratic trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU/L)</td>
<td>11 (4.0)</td>
<td>25 (12)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 (2.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.83 (3.1)</td>
<td>5.39 (0.9)</td>
<td>4.61 (0.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.045</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.51 (0.80)</td>
<td>4.48 (0.80)</td>
<td>4.46 (0.83)</td>
<td>0.880</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.64 (0.62)</td>
<td>2.54 (0.62)</td>
<td>2.51 (0.67)</td>
<td>0.368</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.51 (0.37)</td>
<td>1.53 (0.36)</td>
<td>1.52 (0.34)</td>
<td>0.295</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 (0.52–0.93)</td>
<td>0.75 (0.59–1.21)</td>
<td>0.76 (0.59–1.11)</td>
<td>0.091&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxidized LDL (mU/mg)</td>
<td>66 (20)</td>
<td>60 (15)</td>
<td>75 (29)</td>
<td>0.042&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS (μM/L)</td>
<td>3.5 (2.8–7.2)</td>
<td>2.5 (1.3–5.6)</td>
<td>5.1 (3.3–12.4)</td>
<td>0.044</td>
</tr>
<tr>
<td>Hydroxytyrosol (nmol/L)</td>
<td>34 (29–53)</td>
<td>82 (69–136)</td>
<td>49 (34–58)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD) with exception of triglycerides, TBARS, and hydroxytyrosol which are expressed as median (25th–75th percentile).

<sup>a</sup>p < 0.05 versus baseline; <sup>b</sup>p < 0.05 versus 1 h; <sup>c</sup>linear trend.

FIG. 1. Time course of changes in the expression of ALOX5AP, arachidonate 5-lipoxygenase-activating protein; CD36, CD36 molecule (thrombospondin receptor), and OGT, O-linked N-acetylglucosamine (O-GlcNAc) transferase genes after an acute ingestion of 50-mL virgin olive oil. Gene expression is expressed as RQ value (mean ± SEM) calculated using the relative quantification formula (RQ = 2^-ΔACt) where data were normalized first to the endogenous reference gene (GADPH) and after to the untreated control (baseline). *p < 0.05 versus baseline; †p < 0.05 versus 1 h, according to a general linear model. Tukey’s test was used for multiple paired comparisons.

FIG. 2. Time course of changes in the expression of ADAM17, a disintegrin and metallopeptidase domain 17; ADRB2, adrenergic beta-2-receptor; LIAS, lipoic acid synthetase, and PPARBP, PPAR; binding protein genes after an acute ingestion of 50-mL virgin olive oil. Gene expression is expressed as RQ value (mean ± SEM) calculated using the relative quantification formula (RQ = 2^-ΔACt) where data were normalized first to the endogenous reference gene (GADPH) and after to the untreated control (baseline). *p < 0.05 versus baseline, †p < 0.05 versus 1 h, according to a general linear model. Tukey’s test was used for multiple paired comparisons.
relation between hydroxytyrosol values and ADAM17 expression \((r = -0.727, p = 0.027)\).

**Discussion**

In the present study, the time course of changes in the expression of seven insulin sensitivity-related genes in PBMCs of healthy individuals at baseline, 1 and 6 h after 50 mL VOO ingestion was assessed. In a previous work, we observed changes in the expression of these genes after 3 weeks of sustained VOO consumption in the same volunteers. To date, only few nutrigenomics studies have been performed in humans; an important barrier to such studies has been inaccessibility to tissue samples, which is a crucial requirement for gene expression studies (Müller and Kersten, 2003). In this study, we evaluated gene expression in PBMCs directly collected from the CPT™ tubes. Importantly, direct sample collection from the CPT™ tubes ensures rapid cell isolation and prevents gene activation ex vivo. Changes in the expression of OGT, ALOX5AP, and CD36 genes showed a parabolic shape with a different pattern of change at 1 h than at 6 h postprandial (i.e., a quadratic trend) (Fig. 1). A downregulation at 1 h and an upregulation at 6 h after VOO ingestion were observed in OGT expression. ALOX5AP expression was downregulated and that of CD36 was upregulated at 1 h, returning to basal values at 6 h. Changes in the expression of PPARB, LIAS, ADRB2, and ADAM17, followed a linear trend, increasing from 0 to 6 h postprandial, with the highest gene expression observed at 6 h postgestion (Fig. 2). To the best of our knowledge, these findings have not been previously described.

In agreement with previous reports describing the postprandial time course of glucose and insulin, a peak at these parameters was observed at 1 h after VOO ingestion, which returned to baseline values at 6 h postprandial (Axelsen et al., 1999). This positive quadratic trend was similar to that followed by the changes in the expression of CD36. The opposite pattern (negative quadratic trend) was observed for OGT and ALOX5AP gene expression changes. In agreement with this, at 1 h postprandial changes in OGT and ALOX5AP were inversely correlated with the peak of insulin and glucose. Also, an oxidative stress status was present at 6 h postprandial, reflected in an increase in the oxidative damage to lipids and LDL. This observation has been previously noted after single-dose VOO ingestion of 40 mL (Covas et al., 2006) and 50 mL (Fito et al., 2002). The linear increase observed in triglycerides and oxidized LDL from 0 to 6 h, at the postprandial state, was similar to that observed for ADAM17, ADRB2, LIAS, and PPARBP gene expression changes. However, levels of triglycerides and oxidized LDL at 6 h were inversely related with ADAM17 and ADRB2 gene expression changes.

The OGT gene encodes the transferase that catalyzes the addition of a single N-acetylglucosamine to serine or threonine residues. The OGT gene is activated by the insulin signalling (Whelan et al., 2008). Due to the fact that sustained insulin action would be detrimental to physiological homeostasis, several feedback mechanisms are involved in attenuating the signalling of sustained insulin action (Saltiel and Pessin, 2002; Zick, 2005). Among them, the recruitment of OGT from the nucleus to the plasma membrane results in the alteration of key signalling molecules and the attenuation of the insulin signal transduction (Yang et al., 2008). The increase in OGT expression at 6 h after VO ingestion could be related with the above-mentioned feedback mechanisms developed to attenuate the signalling of sustained insulin action (Yang et al., 2008). The expression of ALOX5AP has been associated with insulin resistance (Kaaman et al., 2006). In agreement with this, in our study, changes in ALOX5AP gene expression were related with the peak of glucose and insulin at 1 h postprandial. The mRNA expression of ALOX5AP in the

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**Table 3. Pearson Correlation Analysis Between the Evaluated Genes at 1 H**

<table>
<thead>
<tr>
<th></th>
<th>ADAM17</th>
<th>ADRB2</th>
<th>ALOX5AP</th>
<th>CD36</th>
<th>LIAS</th>
<th>OGT</th>
<th>PPARBP</th>
</tr>
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<tbody>
<tr>
<td>ADAM17</td>
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<td></td>
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<td></td>
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<tr>
<td>ADRB2</td>
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<tr>
<td>ALOX5AP</td>
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<tr>
<td>CD36</td>
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<td>0.837b</td>
<td>0.139</td>
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<tr>
<td>LIAS</td>
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<td>−0.217</td>
<td>0.518</td>
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<td></td>
</tr>
<tr>
<td>OGT</td>
<td>−0.125</td>
<td>−0.094</td>
<td>0.859b</td>
<td>−0.024</td>
<td>−0.139</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PPARBP</td>
<td>0.798b</td>
<td>0.898c</td>
<td>0.208</td>
<td>0.918c</td>
<td>0.471</td>
<td>0.029</td>
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</table>

Data showed the Pearson correlation coefficient \((r)\), \(^{a}p < 0.05\), \(^{b}p < 0.01\), and \(^{c}p < 0.001\).

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**Table 4. Pearson Correlation Analysis Between the Evaluated Genes at 6 H**

<table>
<thead>
<tr>
<th></th>
<th>ADAM17</th>
<th>ADRB2</th>
<th>ALOX5AP</th>
<th>CD36</th>
<th>LIAS</th>
<th>OGT</th>
<th>PPARBP</th>
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<tr>
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<tr>
<td>ADRB2</td>
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<td>ALOX5AP</td>
<td>0.026</td>
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<tr>
<td>CD36</td>
<td>0.676e</td>
<td>0.116</td>
<td>0.135</td>
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<td></td>
<td></td>
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<tr>
<td>LIAS</td>
<td>0.810b</td>
<td>0.523</td>
<td>−0.209</td>
<td>0.634a</td>
<td>1</td>
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<tr>
<td>OGT</td>
<td>0.898c</td>
<td>0.408</td>
<td>−0.249</td>
<td>0.719a</td>
<td>0.941c</td>
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</tr>
<tr>
<td>PPARBP</td>
<td>0.839c</td>
<td>0.483</td>
<td>−0.134</td>
<td>0.621a</td>
<td>0.947c</td>
<td>0.935c</td>
<td>1</td>
</tr>
</tbody>
</table>

Data showed the Pearson correlation coefficient \((r)\), \(^{a}p < 0.05\), \(^{b}p < 0.01\), and \(^{c}p < 0.001\).
subcutaneous adipose tissue has shown to be increased in obesity and normalized following weight reduction (Kaaman et al., 2006). A recent study also concluded that genetic variation in the ALOX5AP gene contributes to CHD risk in patients with familial hypercholesterolemia (van der Net et al., 2008). Soluble CD36 levels have shown to be closely related with insulin resistance (Handberg et al., 2009). However, CD36 deficiency has also been reported to underlie insulin resistance in spontaneously hypertensive rats (Aitman et al., 1999). Despite a similar quadratic trend observed for insulin and CD36 gene expression changes, we did not observe a relationship between both parameters at 1 h postprandial. At this time, the observed upregulation of CD36, a fatty acid transporter (Corpeleijn et al., 2008), could be related with the postprandial increase in plasma fatty acids after the VOO ingestion, as a related mechanism to the fatty acid uptake (Goldberg et al., 2008). The increase in CD36 could also be associated with the satiety response after olive oil ingestion. Oleic acid, the main fatty acid of olive oil, is the substrate for oleoylthanolamide production. CD36 mediates the intake of oleic acid. Interestingly, oleoylthanolamide production has been reported to be disrupted in mice lacking the membrane-fatty acid transported CD36. The activation of the small-intestinal lipid messenger oleoylthanolamide, enabled by CD36-mediated uptake of dietary oleic acid, serves as a molecular sensor linking fat ingestion to satiety (Schwartz et al., 2008). Induction of CD36 in response to these diverse stimuli, including oxidized LDL, is dependent on the activation of PPARγ (Nicholson, 2004). In agreement with this, a direct relationship was observed between CD36 and PPARBP gene expressions at 1 h and 6 h after olive oil ingestion.

Genes presenting a postprandial linear trend pattern of expression changes were comprised of LIAS, PPARBP, ADRB2, and ADAM17. This group of genes was significantly upregulated at 6 h after VOO ingestion. Changes in the expression of LIAS and PPARBP were strongly related at 6 h postprandial. The protein encoded by the LIAS gene belongs to the lipooic acid synthetases family. Lipoic acid is a powerful antioxidant that can activate peroxisome proliferator-activated receptors (PPARs) and PPARγ (Pershadsingh, 2007). PPARγ is a nuclear hormone receptor playing a crucial role in adipogenesis and insulin sensitization (Fajas et al., 2001). The improvement of insulin resistance by PPARγ agonists is primarily mediated by enhancing the PPARγ interaction with PPAR-binding protein (PPARBP) (Fujimura et al., 2006). Increases in aortic O2 production, glucose, and insulin resistance, as well as a decrease in PPARγ protein in aorta and heart tissues, were prevented or attenuated in glucose-treated rats fed with lipoic acid (El Midaoui et al., 2006; Midaoui et al., 2003). Thus, it could be hypothesised that the upregulation in the expression of LIAS, the gene which codifies the lipoic acid synthase, and that of PPARBP, a PPARγ co-activator, could be one of the feedback mechanisms for counteracting the postprandial oxidative stress involved in the development of insulin resistance (Giugliano et al., 2008). Activation of PPARγ by PPARBP may also increase insulin sensitivity by down-regulating the expression of TNFα (Itoh et al., 1999). TNF-α is the major negative regulator of the insulin receptor pathway. TNF-α is regulated at posttranscriptional level by the TNFα-converting enzyme (ADAM17, also known as TACE) (Serino et al., 2007). ADAM17 is considered to be a significant target for controlling insulin resistance (Togashi et al., 2002). In our study, although a direct relationship was expected (given the increase in both oxidized LDL and ADAM17 expression), an inversely correlation was observed between both parameters at 6 h after VOO ingestion. The direct relationship observed among changes in the expression of LIAS, PPARBP, and ADAM17 genes reinforces their involvement in a hypothetical pathway. The inverse relationship observed between the peak in plasma hydroxytyrosol at 1 h after olive oil ingestion and ADAM17 gene expression is in agreement with previous data showing a decrease of TNF-α levels by hydroxytyrosol (Bitler et al., 2005; Gong et al., 2008).

The adrenergic beta-2 receptor (ADRB2) gene encodes for a major lipolytic receptor in human fat cells. B2-agonists can affect glucose homeostasis through the modulation of insulin secretion, glucagon secretion, hepatic glucose production, and uptake of glucose into muscle (Philipson, 2002). A functional expression of β2 adrenergic receptors is considered to be related to a protection against oxidative stress through the promotion of glutathione synthesis (Takahata et al., 2009). From our results, the ADRB2 gene expression was inversely correlated with oxidized LDL and triglycerides at 6 h after VOO ingestion, reinforcing the above-mentioned possible protection. Insulin treatment has shown to increase ADRB2 protein expression in old rats (Paulose and Balakrishnan, 2008). Thus, the ADRB2 upregulation observed at 6 h VOO ingestion could be promoted by the postprandial insulin peak. This could count for a delayed adrenergic signal for both adipocyte differentiation and oxidative stress protection (Takahata et al., 2009).

Gene expression changes in insulin sensitivity related genes were observed after a single dose of 50 mL (44.5 g) of VOO, a quantity reported (from 30 g to 50 g/day) as usual in the Mediterranean diet (Helsing, 1995). The impact of different doses of olive oil on the gene expression patterns of insulin sensitivity-related genes remains unknown. Lesser doses of olive oil could reduce the expression of genes related with the fatty uptake or postprandial oxidative stress, such as CD36, PPARBP, ADRB2, or LIAS. Because the oral fat load used in the present study was VOO, it is still unknown whether the same gene response could be extrapolated after 50 mL of other types of fats. In one of our previous works (Covas et al., 2006), phenolic compounds from olive oil were able to modulate the postprandial oxidative stress. Thus, phenolic compounds present in the VOO administered could account for a differential response in oxidative stress related genes. In our past experience (Khymenets et al., 2009), all the genes referred to in this study increased their expression after 3 weeks of sustained (25 mL/day) olive oil consumption, the strongest responders being ADAM17, CD36, LIAS, OGT, and PPARBP (p < 0.001). The aim of the present study was to compare changes in gene expression associated with olive oil ingestion at different postprandial states. Although the subjects’ baseline data had served as a within-subject control, a limitation of the study is, however, the lack of control group for the intervention itself. Due to this, the observed effects on gene expression could be secondary, not only due to the VOO ingestion, but also due to a time course effect on circadian regulated genes (Khymenets et al., 2008) and to physiological changes following any fat meal intake. It remains to be determined in future studies how specific the expression changes of this small set of genes are, compared to other global changes in human gene expression.
In summary, significant changes in the expression of candidate genes related to insulin sensitivity do occur in human PBMNCs after an oral fat load of 50 mL of VOO. Changes in gene expression were modest, as was expected after administration of a real-life dose of a single food, such as raw VOO. In terms of future clinical nutrigenomic study designs, it is useful to know that a single dose of VOO can elicit changes in gene expression in targets that are mechanistically relevant for metabolic syndrome. The exact mechanisms underlying the down- or upregulation of insulin sensitivity-related genes occurring after the oral raw VOO load, their protein expression, and their associations with changes in insulin sensitivity merit further investigation.

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