OMICS A Journal of Integrative Biology
Volume 13, Number 5, 2009

DOI: 10.1089/omi.2008.0085

Original Article

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 Fito[1]

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 ± 0.32) and arachidonate-5-lipoxygenase-activating protein (*ALOX5AP*, RQ: 0.64 ± 0.31) genes ($p < 0.005$). *OGT* was upregulated at 6 h (RQ: 1.88 ± 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

[1]Cardiovascular Risk and Nutrition Research Group and [2]Human Pharmacology and Clinical Neurosciences Research Group, Institut Municipal d'Investigació Mèdica (IMIM-Hospital del Mar), CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Barcelona, Spain.
[3]Departament de Ciències Experimentals i de la Salut, Pompeu Fabra University (CEXS-UPF), Barcelona, Spain.
[4]Universitat Autonoma de Barcelona, Barcelona, Spain.

atherosclerosis-related responsive genes observed in peripheral blood mononuclear cells (PBMNCs) 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 metallopeptidase 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 *PPARBP*, 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). Notewithstanding 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 PBMNCs, 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/m^2. 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 PBMNCs using the Ultraspec solution (Biotecx Laboratories, Houston, TX) was utilized. In brief, PBMNCs were isolated from peripheral blood using BD Vacutainer® CPT™ cell preparation

tubes (Beckton Dickinson). Whole blood was centrifuged at 1690×*g* for 30 min; cells were washed with phosphate-buffered saline (PBS), centrifuged at 970×*g* for 15 min, resuspended in Ultraspec, and finally stored at −80°C until RNA isolation. Total RNA (tRNA) was extracted from the Ultraspec solution following manufacturers' instructions. Quality and purity of total RNA was obtained. Total RNA concentration and tRNA purity (ratios A260/A280 and A260/A230) were estimated by spectrophotometry (NanoDrop® ND-1000, NanoDrop Technologies, Wilmington, DE). tRNA integrity was assessed by micro capillary gel electrophoresis with fluorescent detection (Bioanalyzer, RNA 6000 LabChip kit, Agilent Technologies, Wilmington, DE), and was estimated by the RIN value (RNA integrity number) (Fleige and Pfaffl, 2006). The RIN value was determined by the Agilent 2100 Expert Software algorithm (Agilent Technologies, Palo Alto, CA). All reagents, plastic ware, and supplies used were sterile, nuclease free, and of molecular biology grade.

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 metallopeptidase 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) are 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 baseline 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 $8.5 \leq \text{RIN} \leq 9.5$).

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 ($\text{RQ} = 1.6 \pm 0.8$, $p < 0.05$) 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 ($p < 0.05$). Several

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

Gene symbol	*Gene name*	*Assay ID*	*Context sequence*
ADAM17	ADAM metallopeptidase domain 17 (TACE,Tumour necrosis factor alpha converting enzyme)	Hs00234224_m1	TGTCCAGTGCAGTGACAGGAACAGT
ADRB2	Adrenergic beta-2-receptor	Hs00240532_s1	CCACCCACCAGGAAGCCATCAACTG
ALOX5AP	Arachidonate 5-lipoxygenase-activating protein	Hs00233463_m1	ACTGCCAACCAGAACTGTGTAGATG
CD36	CD36 molecule (thrombospondin receptor)	Hs00169627_m1	ATGATTAATGGTACAGATGCAGCCT
LIAS	Lipoic acid synthetase	Hs00398048_m1	CATATAAAGCAGGTGAATTTTTCCT
OGT	O-linked N-acetylglucosamine (GlcNAc) transferase	Hs00269228_m1	ACTTTGAAAAGGCTGTCACCCTTGA
PPARBP	PPARg binding protein	Hs00191130_m1	GGGGAGAATCCTGTGAGCTGTCCGG

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

Parameters	*Baseline (0 hours)*	*1 hour*	*6 hours*	*p value for quadratic trend*
Insulin (mU/L)	11 (4.0)	25 (12)[a]	7.5 (2.3)[b]	0.001
Glucose (mmol/L)	4.83 (3.1)	5.39 (0.9)	4.61 (0.3)[b]	0.045
Total cholesterol (mmol/L)	4.51 (0.80)	4.48 (0.80)	4.46 (0.83)	0.880
LDL cholesterol (mmol/L)	2.64 (0.62)	2.54 (0.62)	2.51 (0.67)	0.368
HDL cholesterol (mmol/L)	1.51 (0.37)	1.53 (0.36)	1.52 (0.34)	0.295
Triglycerides (mmol/L)[a]	0.65 (0.52–0.93)	0.75 (0.59–1.21)	0.76 (0.59–1.11)	0.091[c]
Oxidized LDL (mU/mg LDL cholesterol)	66 (20)	60 (15)	75 (29)	0.042[c]
TBARS (μM/L)	3.5 (2.8–7.2)	2.5 (1.3–5.6)	5.1 (3.3–12.4)	0.044
Hydroxytyrosol (nmol/L)	34 (29–53)	82 (69–136)	49 (34–58)	0.003

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

[a]$p < 0.05$ versus baseline; [b]$p < 0.05$ versus 1 h; [c]linear trend.

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* infraexpression ($r = -0.685$, $p = 0.020$) 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* upregulation 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 cor-

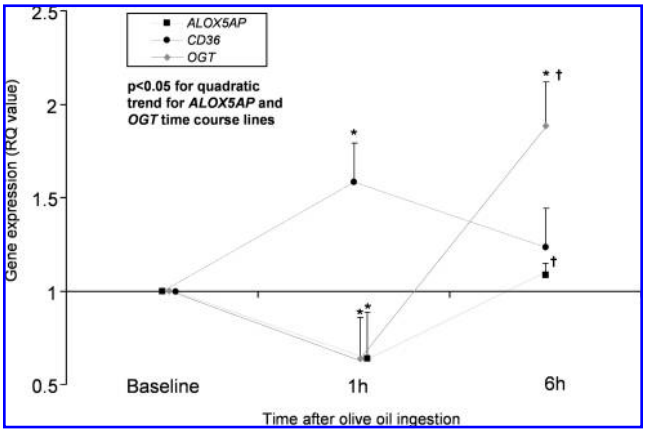

FIG. 1. Time course of changes in the expression of *ALOX5AP*, arachidonate 5-lipoxygenase-activating protein; *CD36*, CD36 molecule (thrombospondin receptor), and *OGT*, *O*-linked N-acetylglucosamine (*O*-GlcNAc) transferase genes after an acute ingestion of 50-mL virgin olive oil. Gene expression is expressed as RQ value (mean ± SEM) calculated using the relative quantification formula ($RQ = 2^{-\Delta\Delta Ct}$) 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.

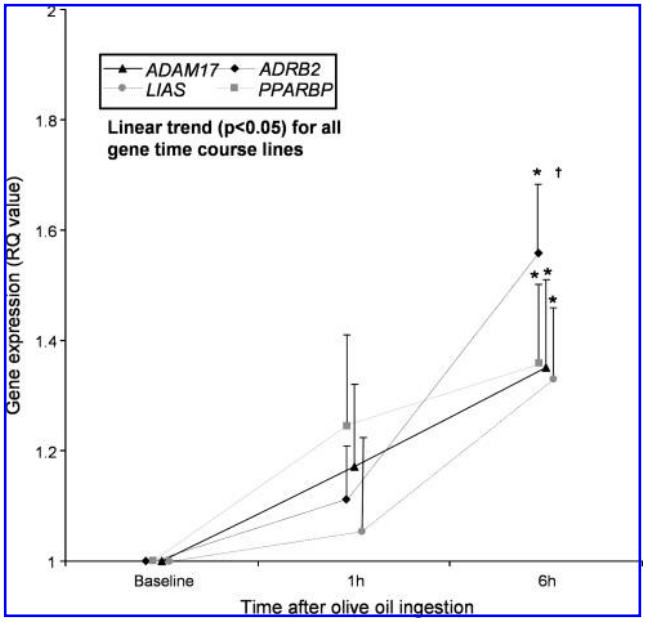

FIG. 2. Time course of changes in the expression of *ADAM17*, a disintegrin and metallopeptidase domain 17; *ADRB2*, adrenergic beta-2-receptor; *LIAS*, lipoic acid synthetase, and *PPARBP*, PPARγ binding protein genes after an acute ingestion of 50-mL virgin olive oil. Gene expression is expressed as RQ value (mean ± SEM) calculated using the relative quantification formula ($RQ = 2^{-\Delta\Delta Ct}$) 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.

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

	ADAM17	*ADRB2*	*ALOX5AP*	*CD36*	*LIAS*	*OGT*	*PPARBP*
ADAM17	1						
ADRB2	0.748[b]	1					
ALOX5AP	−0.050	0.185	1				
CD36	0.728[a]	0.837[b]	0.139	1			
LIAS	0.410	0.370	−0.217	0.518	1		
OGT	−0.125	−0.094	0.859[b]	−0.024	−0.139	1	
PPARBP	0.798[b]	0.898[c]	0.208	0.918[c]	0.471	0.029	1

Data showed the Pearson correlation coefficient (r), [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$.

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 PBMNCs 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 (Muller and Kersten, 2003). In this study, we evaluated gene expression in PBMNCs 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*, *ABRB2*, and *ADAM17*, followed a linear trend, increasing from 0 to 6 h postprandial, with the highest gene expression observed at 6 h postingestion (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 in 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 VOO 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

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

	ADAM17	*ADRB2*	*ALOX5AP*	*CD36*	*LIAS*	*OGT*	*PPARBP*
ADAM17	1						
ADRB2	0.522	1					
ALOX5AP	0.026	0.057	1				
CD36	0.676[a]	0.116	0.135	1			
LIAS	0.810[b]	0.523	−0.209	0.634[a]	1		
OGT	0.898[c]	0.408	−0.249	0.719[a]	0.941[c]	1	
PPARBP	0.839[c]	0.483	−0.134	0.621[a]	0.947[c]	0.935[c]	1

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 oleoylethanolamide production. *CD36* mediates the intake of oleic acid. Interestingly, oleoylethanolamide production has been reported to be disrupted in mice lacking the membrane-fatty acid transported *CD36*. The activation of the small-intestinal lipid messenger oleoylethanolamide, 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*, *ABRB2*, 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 lipoic acid synthetases family. Lipoic acid is a powerful antioxidant that can activate peroxisome proliferator-activated receptors (*PPARα* 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 O_2^- 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 downregulating 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 inversed correlation was observed between both parameters at 6 h after VOO ingestion. The direct relationship observed among changes in the expression of *LIAS*, *PPARB*, 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 *β*2 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.

Acknowledgments

The CIBEROBN is an initiative of the Instituto de Salud Carlos III, Madrid, Spain. This work has been supported by CICYT (SAF 2004-08173-CO3-00), by SNS contract (CP06/00100) Instituto de Salud Carlos III, by the Greek State Scholarship Foundation (I.KY. Athens, Greece), and partially supported by The Generalitat of Catalunya (2005 SGR 00577).

Author Disclosure Statement

No competing financial interests exist.

References

Aitman, T.J., Glazier, A.M., Wallace, C.A., Cooper, L.D., Norsworthy, P.J., Wahid, F.N., et al. (1999). Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. Nat Genet 21, 76–83.

Axelsen, M., Smith, U., Eriksson, J.W., Taskinen, M.R., and Jansson, P.A. (1999). Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. Ann Intern Med 131, 27–31.

Berge, R.K., Tronstad, K.J., Berge, K., Rost, T.H., Wergedahl, H., Gudbrandsen, O.A., et al. (2005). The metabolic syndrome and the hepatic fatty acid drainage hypothesis. Biochimie 87, 15–20.

Bitler, C.M., Viale, T.M., Damaj, B., and Crea, R. (2005). Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. J Nutr 135, 1475–1479.

Ceriello, A. (1999). Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Diabetes Nutr Metab 12, 42–46.

Ceriello, A. (2000). Oxidative stress and glycemic regulation. Metabolism 49, 27–29.

Corpeleijn, E., Pelsers, M.M., Soenen, S., Mensink, M., Bouwman, F.G., Kooi, M.E., et al. (2008). Insulin acutely upregulates protein expression of the fatty acid transporter CD36 in human skeletal muscle in vivo. J Physiol Pharmacol 59, 77–83.

Covas, M.I., de la Torre, K., Farre-Albaladejo, M., Kaikkonen, J., Fito, M., Lopez-Sabater, C., et al. (2006). Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radic Biol Med 40, 608–616.

El Midaoui, A., Wu, L., Wang, R., and de Champlain, J. (2006). Modulation of cardiac and aortic peroxisome proliferator-activated receptor-gamma expression by oxidative stress in chronically glucose-fed rats. Am J Hypertens 19, 407–412.

Fajas, L., Debril, M.B., and Auwerx, J. (2001). PPAR gamma: an essential role in metabolic control. Nutr Metab Cardiovasc Dis 11, 64–69.

Fito, M., Gimeno, E., Covas, M.I., Miro, E., Lopez-Sabater mdel, C., Farre, M., et al. (2002). Postprandial and short-term effects of dietary virgin olive oil on oxidant/antioxidant status. Lipids 37, 245–251.

Fleige, S., and Pfaffl, M.W. (2006). RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 27, 126–139.

Friedewald, W.T., Levv, R.I., and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18, 499–502.

Fujimura, T., Kimura, C., Oe, T., Takata, Y., Sakuma, H., Aramori, I., et al. (2006). A selective peroxisome proliferator-activated receptor gamma modulator with distinct fat cell regulation properties. J Pharmacol Exp Ther 318, 863–871.

Giugliano, D., Ceriello, A., and Esposito, K. (2008). Glucose metabolism and hyperglycemia. Am J Clin Nutr 87, 217S–222S.

Goldberg, R.J., Yarzebski, J., Spencer, F.A., Zevallos, J.C., Lessard, D., and Gore, J.M. (2008). Thirty-year trends (1975–2005) in the magnitude, patient characteristics, and hospital outcomes of patients with acute myocardial infarction complicated by ventricular fibrillation. Am J Cardiol 102, 1595–1601.

Gong, D., Geng, C., Jiang, L., Cao, J., Yoshimura, H., and Zhong, L. (2008). Effects of hydroxytyrosol-20 on carrageenan-induced acute inflammation and hyperalgesia in rats. Phytother Res.

Handberg, A., Lopez-Bermejo, A., Bassols, J., Vendrell, J., Ricart, W., and Fernandez-Real, J.M. (2009). Circulating soluble CD36 is associated with glucose metabolism and interleukin-6 in glucose-intolerant men. Diab Vasc Dis Res 6, 15–20.

Helsing, E. (1995). Traditional diets and disease patterns of the Mediterranean, circa 1960. Am J Clin Nutr 61, 1329S–1337S.

Henriksen, E.J. (2006). Exercise training and the antioxidant alpha-lipoic acid in the treatment of insulin resistance and type 2 diabetes. Free Radic Biol Med 40, 3–12.

Itoh, H., Doi, K., Tanaka, T., Fukunaga, Y., Hosoda, K., Inoue, G., et al. (1999). Hypertension and insulin resistance: role of peroxisome proliferator-activated receptor gamma. Clin Exp Pharmacol Physiol 26, 558–560.

Kaaman, M., Ryden, M., Axelsson, T., Nordstrom, E., Sicard, A., Bouloumie, A., et al. (2006). ALOX5AP expression, but not gene haplotypes, is associated with obesity and insulin resistance. Int J Obes (Lond) 30, 447–452.

Khymenets, O., Ortuno, J., Fito, M., Covas, M.A., Farre, M., and de la Torre, R. (2005). Evaluation of RNA isolation procedures from human blood and its application for gene expression studies (Sod-1, Sod-2). Anal Biochem 347, 156–158.

Khymenets, O., Covas, M.I., Farre, M., Langohr, K., Fito, M., and de la Torre, R. (2008). Role of sex and time of blood sampling in SOD1 and SOD2 expression variability. Clin Biochem 41, 1348–1354.

Khymenets, O., Fito, M., Covas, M.I., Farre, M., Pujadas, M.A, Muñoz, D., et al. (2009). Mononuclear cell transcriptome response after sustained virgin olive oil consumption in humans: an exploratory study. OMICS (in press).

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.

Midaoui, A.E., Elimadi, A., Wu, L., Haddad, P.S., and de Champlain, J. (2003). Lipoic acid prevents hypertension, hyperglycemia, and the increase in heart mitochondrial superoxide production. Am J Hypertens 16, 173–179.

Miro-Casas, E., Covas, M.I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., et al. (2003). Hydroxytyrosol disposition in humans. Clin Chem 49, 945–952.

Muller, M., and Kersten, S. (2003). Nutrigenomics: goals and strategies. Nat Rev Genet 4, 315–322.

Nicholson, A.C. (2004). Expression of CD36 in macrophages and atherosclerosis: the role of lipid regulation of PPARgamma signaling. Trends Cardiovasc Med 14, 8–12.

Paulose, C.S., and Balakrishnan, S. (2008). Adrenergic, dopaminergic and serotonergic gene expression in low dose, long time insulin and somatotropin treatment to ageing rats: rejuvenation of brain function. Biogerontology 9, 429–439.

Pedersen, O. (1999). Genetics of insulin resistance. Exp Clin Endocrinol Diabetes 107, 113–118.

Pershadsingh, H.A. (2007). Alpha-lipoic acid: physiologic mechanisms and indications for the treatment of metabolic syndrome. Expert Opin Investig Drugs 16, 291–302.

Philipson, L.H. (2002). Beta-Agonists and metabolism. J Allergy Clin Immunol 110, S313–317.

Pyorala, M., Miettinen, H., Laakso, M., and Pyorala, K. (1998). Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. Circulation 98, 398–404.

Regnstrom, J., Nilsson, J., Tornvall, P., Landou, C., and Hamsten, A. (1992). Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. Lancet 339, 1183–1186.

Riserus, U. (2008). Fatty acids and insulin sensitivity. Curr Opin Clin Nutr Metab Care 11, 100–105.

Roche, H.M., and Gibney, M.J. (2000). The impact of postprandial lipemia in accelerating atherothrombosis. J Cardiovasc Risk 7, 317–324.

Salati, L.M., Szeszel-Fedorowicz, W., Tao, H., Gibson, M.A., Amir-Ahmady, B., Stabile, L.P., et al. (2004). Nutritional regulation of mRNA processing. J Nutr 134, 2437S–2443S.

Saltiel, A.R., and Pessin, J.E. (2002). Insulin signaling pathways in time and space. Trends Cell Biol 12, 65–71.

Schwartz, G.J., Fu, J., Astarita, G., Li, X., Gaetani, S., Campolongo, P., et al. (2008). The lipid messenger OEA links dietary fat intake to satiety. Cell Metab 8, 281–288.

Serino, M., Menghini, R., Fiorentino, L., Amoruso, R., Mauriello, A., Lauro, D., et al. (2007). Mice heterozygous for tumor necrosis factor-alpha converting enzyme are protected from obesity-induced insulin resistance and diabetes. Diabetes 56, 2541–2546.

Steiner, G., and Lewis, G.F. (1996). Hyperinsulinemia and triglyceride-rich lipoproteins. Diabetes 45 (Suppl 3), S24–S26.

Stout, R.W. (1996). Hyperinsulinemia and atherosclerosis. Diabetes 45 (Suppl 3), S45–S46.

Takahata, Y., Takarada, T., Iemata, M., Yamamoto, T., Nakamura, Y., Kodama, A., et al. (2009). Functional expression of beta2 adrenergic receptors responsible for protection against oxidative stress through promotion of glutathione synthesis after Nrf2 upregulation in undifferentiated mesenchymal C3H10T1/2 stem cells. J Cell Physiol 218, 268–275.

Togashi, N., Ura, N., Higashiura, K., Murakami, H., and Shimamoto, K. (2002). Effect of TNF-alpha-converting enzyme inhibitor on insulin resistance in fructose-fed rats. Hypertension 39, 578–580.

van der Net, J.B., Versmissen, J., Oosterveer, D.M., Defesche, J.C., Yazdanpanah, M., Aouizerat, B.E., et al. (2008). Arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene and coronary heart disease risk in familial hypercholesterolemia. Atherosclerosis.

Weinbrenner, T., Fito, M., de la Torre, R., Saez, G.T., Rijken, P., Tormos, C., et al. (2004). Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. J Nutr 134, 2314–2321.

Whelan, S.A., Lane, M.D., and Hart, G.W. (2008). Regulation of the O-linked beta-N-acetylglucosamine transferase by insulin signaling. J Biol Chem 283, 21411–21417.

Williams, S.B., Goldfine, A.B., Timimi, F.K., Ting, H.H., Roddy, M.A., Simonson, D. C., et al. (1998). Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation 97, 1695–1701.

Yang, X., Ongusaha, P.P., Miles, P.D., Havstad, J.C., Zhang, F., So, W.V., et al. (2008). Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. Nature 451, 964–969.

Zavaroni, I., Bonini, L., Gasparini, P., Barilli, A.L., Zuccarelli, A., Dall'Aglio, E., et al. (1999). Hyperinsulinemia in a normal population as a predictor of non-insulin-dependent diabetes mellitus, hypertension, and coronary heart disease: the Barilla factory revisited. Metabolism 48, 989–994.

Zick, Y. (2005). Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. Sci STKE 2005, pe4.

Address correspondence to:
Maria-Isabel Covas, DPharm, Ph.D.
Cardiovascular Risk and Nutrition Research Group
Institut Municipal d'Investigació Mèdica
(IMIM-Hospital del Mar)
Parc de Recerca Biomèdica de Barcelona (PRBB)
Carrer Dr. Aiguader, 88. 08003, Barcelona, Spain

E-mail: mcovas@imim.es