REVIEW

Up-to date knowledge on the in vivo transcriptomic effect of the Mediterranean diet in humans

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The present review discusses and summarizes the up-to-date body of knowledge concerning human nutrigenomic studies with Mediterranean diet (MedDiet) and olive oil (OO) interventions, at real-life doses and conditions. A literature review was carried out until March 2012. Original articles assessing the nutrigenomic effect of the MedDiet and its main source of fat, OO, on gene expression were selected. State-of-the-art data in this field, although scarce, are promising. Despite a great diversity among studies, the attributed health benefits of the MedDiet and its components, such as OO, could be explained by a transcriptomic effect on atherosclerosis, inflammation, and oxidative stress-related genes (i.e. *ADRB2*, *IL7R*, *IFN*γ, *MCP1*, *TNF*α). Gene expression changes toward a protective mode were often associated with an improvement in systemic markers for oxidation and inflammation. The suggested underlying molecular pathways responsible for these changes, and the extent to which evidence exists of a MedDiet and OO nutrigenomic effect, are also discussed.

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Abbreviations: **ADAM17**, ADAM metallopeptidase domain 17; **ADRB2**, adrenoreceptor beta 2; **ALDH1A1**, aldehyde dehydrogenase 1 family, member A1; **ALOX5AP**, arachidonate 5-lipoxygenase-activating protein; **ARHGAP15**, Rho GTPase activating protein 15; **BiP/Grp78**, glucose-regulated protein, 78kDa; **BIRC1**, NLR family, apoptosis inhibitory protein; **CCL5/RANTES**, chemokine (C-C motif) ligand 5; **CD40/CD40L**, CD40 antigen ligand; **COX2**, cyclooxigenase-2; **ERCC5**, DNA excision repair protein; **GLUT4**, glucose transporter 4; **JNK1**, Jun N-terminal kinase 1; **IKB**α, NF-kappa-B inhibitor alpha; **IKKb**, inhibitor of nuclear factor kappa-B kinase subunit beta; **IL7R**, interleukin 7 receptor; **IFN**γ, interferon gamma; **KEAP1**, kelch-like ECH-associated protein 1; **LEP**, leptin; **LIAS**, lipoic acid synthetase; **LPL**, lipoprotein lipase; **LRP1**, low-density lipoprotein receptor-related protein; **MCP1**, monocyte chemoattractant protein 1; **MedDiet**, Mediterranean diet; **MMP9**, matrix metallopeptidase 9; **NF**κ**B**, nuclear factor NF-kappa-B; **NRF2**, nuclear factor (erythroid-derived 2)-like 2; **OGT**, O-linked *N*-acetylglucosamine (GlcNAc) transferase; **PBMCs**, peripheral blood mononuclear cells; **PPARBP**, peroxisome proliferator-activated receptor-binding proteína; **POLK**, polymerase κ; **p22^{phox} and p47^{phox}**, NADPH oxidase subunits; **RAC2**, rho family, small GTP binding protein Rac2; **SOD1**, superoxide dismutase 1; **SOD2**, superoxide dismutase 2; **sXBP1**, X-box binding protein 1; **TNF**α, tumor necrosis factor α; **TNFSF10**, tumor necrosis factor (ligand) superfamily, member 10; **TRXR**, thioredoxin reductase; **USP48**, ubiquitin-specific peptidase 48; **VOO**, virgin olive oil; **XRCC5**, x-ray repair complementing defective repair

1 Introduction

Since the 1960s, the Mediterranean diet (MedDiet) refers to dietary patterns found in olive-growing areas of the Mediterranean region [1–3]. The MedDiet is a primordial dietary pattern, which consists of diet variants depending on each region in the Mediterranean basin. All MedDiet variants may have their own peculiarities, but olive oil (OO) is considered the hallmark of this dietary pattern and its main source of fat [2]. The MedDiet is characterized by (i) a high consumption of vegetables, legumes, fruits, and cereals; (ii) a regular but moderate wine intake; (iii) moderate consumption of fish; (iv) low consumption of meat; and (v) low-to-moderate intake of dairy products [3]. Total lipid intake may be high, around or in

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excess of 40% of total energy intake as in Greece, or moderate, around 30% of total energy intake, as in Italy [2]. The ratio of MUFAs to saturated fatty acids (SFAs) is much higher in the regions where a MedDiet pattern is followed than in other places in the world [4]. Besides being a dietary pattern, the MedDiet is associated with a concrete lifestyle with a moderate-to-high level of physical activity and the daily, high consumption of water [5].

OO is a functional food, which in addition to having a high level of MUFAs, also contains multiple minor components with biological properties. The content of the minor components of an OO varies depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system employed. Different processing methods produce virgin, ordinary, or pomace OO [6]. Virgin OO (VOO) is produced by direct pressing or centrifugation of the olives and it is rich in phenolic compounds (around 150–400 ppm in those generally present in the market). VOO with an acidity greater than 3.0° (2.0 in EU) are submitted to a refining process in which some components, mainly phenolic compounds and to a lesser degree squalene, are lost [7]. By mixing virgin and refined OO, an ordinary OO, with a lower phenolic content (around 50–150 ppm) (EU 1991) is produced and marketed. After VOO production, the rest of the olive drupe and seed is processed and submitted to refining process, after which a certain quantity of VOO is added before marketing. The resulting product is pomace OO, with a low phenolic content (around 10–70 ppm).

In 2010, the UNESCO included the MedDiet on the representative list of the Intangible Cultural Heritage of Humanity [http://www.unesco.org/culture/ich/en/RL/00394]. Adherence to the MedDiet has been associated with a reduced risk of overall and cardiovascular mortality, cancer incidence and mortality, and incidence of Parkinson's and Alzheimer's disease [4, 8, 9]. The most impressive benefits of this diet, however, are related to reductions in cardiovascular morbidity and mortality [10]. Recent analyses suggest that, together with regular physical activity and smoking cessation, over 80% of coronary heart disease (CHD), 70% of stroke, and 90% of type 2 diabetes can be avoided by healthy food choices that are consistent with the traditional MedDiet (TMD) [11]. It has also been demonstrated that the MedDiet and OO consumption are effective in reducing classical and novel risk factors for CHD [12, 13]. A striking protective effect of a Mediterranean-type diet (rich in alpha-linolenic acid) was also reported in the Lyon Diet Heart Study, with a 50–70% reduction in the risk of recurrence, after 4 years of follow-up, in CHD patients. This study provided the highest level of evidence to recommend a Mediterranean-type diet as a useful tool in the secondary prevention of CHD [14]. The task at hand is to corroborate the results of the Lyon Diet Heart Study in both primary and secondary CHD prevention. This goal is now on-going through the development of the PREDIMED Study, a large multicenter Spanish study aimed to assess the effect of the MedDiet on the primary prevention of cardiovascular disease (CVD) [15].

1.1 The MedDiet in the nutrigenomic era

Among the mechanisms through which the MedDiet and its main source of fat, OO, exert their beneficial effects on human health, gene–diet interactions could play an important role in the development of and in the protection against chronic degenerative diseases. From a nutrigenomic point of view, nutrients act as dietary signals, are detected by the cellular sensor, and influence gene and protein expression and, subsequently, metabolite production [16]. In the nutrigenomic era, attention is drawn to the importance of genes in human nutrition, and the nutritional field has started to focus on molecular changes. The mechanism of action of nutrients in the human organism is strongly related to their capacity to modulate gene expression. Expression profile "signatures," defined as the characteristic patterns of differential gene expression, can now be used for the development of novel biomarkers after exposure to different levels of nutrients [17].

On the basis of the precepts of evidence-based medicine, a high level (I or II) of scientific evidence is required before nutritional recommendations, for the general population, can be formulated. Randomized, controlled, double-blind, clinical intervention trials are the ones which can provide the required scientific evidence (level I of evidence) and, to some extent, large cohort studies (level II of evidence) can also do so [18]. These types of study designs provide the best approach for conducting gene–nutrient phenotype association studies [19]. Moreover, high-throughput techniques are promising tools to unravel the complex biological networks specifically underlying chronic degenerative diseases [20].

Nutrigenomics embrace all "omics" fields, such as genomics, transcriptomics, proteomics, and metabolomics with the aim of understanding and characterizing how nutrients and/or food act at molecular level. Thanks to the rapid evolution of these high-throughput "omics" technologies, we are now able to generate and analyze large-scale data on DNA, RNA, protein, and metabolites. The combination of "omics" studies is believed to help clarify the mechanisms of action by which the MedDiet exerts its beneficial effects on human health. In human nutrigenomic studies, peripheral blood mononuclear cells (PBMCs) are mainly selected to explore changes in gene expression for their availability [21]. PBMCs are (i) critically involved in atherosclerotic plaque formation, because of their possible roles in the development of inflammation (cytokine production), immunity, and lipid regulation processes; (ii) easily available from volunteers, considering the feasibility of collection plus deontological reasons; and (iii) their collection can be done directly, i.e. from BD Vacutainer® CPT™ tubes or Ficoll-Paque™ PLUS, thus ensuring rapid isolation and avoiding ex vivo gene activation [22]. PBMC is a general term including monocytes and lymphocytes. All these cells are involved in the atherosclerotic process. Monocytes are key cells in all its phases from the formation of foam cells to the destabilization and rupture of the atherosclerotic plaque [23]. B cells have a role in directing the immune

response during atherosclerosis, and T cells recruitment in the arterial adventitia is accelerated in the atherosclerosis development [24]. In the present work, we focus on reviewing the current knowledge on the transcriptomic effect of the MedDiet, as a whole dietary pattern, and that of OO and its polyphenols, in human studies.

2 Transcriptomic effect of the MedDiet and OO: Human trials

A literature review was carried out in MEDLINE until March 2012. We searched for clinical studies, assessing the effect either of acute (single dose) or sustained MedDiet or OO consumption on human gene expression. Cohort studies aimed at assessing the relationship between adherence to the MedDiet and transcriptomic effects were also looked for, but none was obtained. The following Medical Subject Heading Terms: *Mediterranean diet, olive oil, polyphenols, gene expression, transcriptomics, human, healthy, in vivo, patients, postprandial*, and *sustained consumption* were used. Our primary aim was to perform meta-analyses, or a systemic review, based on the PRISMA criteria [25], with all the studies involved. However, the small number of available studies, the lack of homogeneity among the study designs, and the differences in end point variables (i.e. gene expression), did not permit such an approach. Studies performed in cellular or animal models, although found, were beyond the scope of the present review. Thus, they were not included for discussion. As a result, a critical review of the 14 original studies found was finally performed. Table 1 summarizes the results obtained in human nutrigenomic studies conducted with the MedDiet and the OO, both at postprandial time and after sustained consumption.

2.1 Transcriptomic effect of the MedDiet

During 2011, three studies reported transcriptomic results after MedDiet, at postprandial or at sustained consumption (Table 1). In a randomized, crossover study, the postprandial inflammatory response in PBMCs, after a 3-week consumption of three diets, was assessed in 20 elderly individuals [26]. A lower postprandial expression of genes involved in inflammatory processes was observed when the MedDiet was compared with (i) SFA-rich diet (*NFκB p65 subunit, MCP1,* and *MMP9*) or (ii) a high carbohydrate n3-PUFA enriched diet (*NFκB p65 subunit* and *TNFα*). In both situations, *IκBa*, the inhibitor of κB involved in *NFκB* inactivation, was comparatively upregulated after MedDiet [26]. After SFA-diet an increase in plasma levels of *MCP1* was also observed. A postprandial increase in *IL6* and decrease in TNFα plasma levels was observed after all diets consumed [26].

Oxidation and inflammation are intertwined processes. In agreement with the results referred to before, a lower postprandial expression of oxidative stress-related genes such as *NRF2, p22^{phox}, p47^{phox}, SOD1, SOD2, and TRXR* was observed after 4 weeks of two MedDiets (with and without CoQ) versus a SFA-rich diet in elderly individuals [27]. Citoplasmatic levels of NRF2 and Keap proteins were, however, higher after the MedDiets. Within this study, the effect of the two MedDiets on inflammation-related genes was also assessed [27]. As expected, there was a decrease in the expression of inflammatory genes such as *IL1B, JNK1, p65, IKK-b, MMP9, IL1B, JNK1, SXBP-1, and BiP/Grp78* genes and a higher expression in *IKB-a* gene [28] after the MedDiets versus the SFA-rich diet. The transcriptomic effect of the Mediterranean-style low-glycemic-load diet (Medstyle) was also assessed in women with metabolic syndrome and elevated LDL-cholesterol levels [29]. Two MedDiets (with and without a beverage containing soy protein, plant sterols, rho iso-alpha acids, and Acacia proanthocyanidins) were followed during 12 weeks. When results of both arms of the study were joined a decrease in the 3-hydroxy-3-methylglutaryl-coenzyme A (*HMB-CoA*) reductase gene expression, a key regulatory gene of cholesterol synthesis, in PBMC was observed which directly correlated with a decrease in plasma insulin [29].

2.2 Transcriptomic effect of OO and its polyphenols

Among all the MedDiet food components, OO, its main source of fat, is the best studied. The high quantities of vegetables and legumes consumed in the MedDiet are generally accompanied by OO. Due to this, it is difficult to disentangle its effects per se, and those of its polyphenols, from those of the MedDiet as a whole [30]. This applies particularly when intervention and control groups are not matched with MedDiet, with and/or without OO or with polyphenol-rich/polyphenol-poor OO. OO, besides having a high level of MUFA, also contains multiple bioactive minor components able to promote health. These minor components of OO are classified into the unsaponifiable and the soluble fraction. The former is defined as the solvent-extracted fraction after the saponification of the oil, whereas the latter includes the polyphenols [31]. In November 2011, the European Food Safety Authority (EFSA) released a claim concerning the benefits derived from the daily ingestion of 5 mg of OO polyphenols (hydroxytyrosol, tyrosol, and their conjugated forms) on protecting LDL from oxidation [http://www.efsa.europa.eu/en/efsajournal/pub/2033.htm]. A key study, supporting this health claim, was the EUROLIVE one [12], which provided evidence of the in vivo protective role of OO polyphenols on lipid oxidative damage, at real-life doses, in healthy humans. The EUROLIVE study concluded that OO polyphenols can account for greater benefits on blood lipids and oxidative damage than those provided by the MUFA and other minor components of OO. Nowadays, it is becoming more evident that polyphenols exert their cellular protection by interacting with intracellular signaling pathways involved in pathological processes [32].

Table 1. Human postprandial and sustained consumption nutrigenomic studies with Mediterranean diet and olive oil

Type of study	Participants	Intervention	Tissue	Measured by	Upregulated genes (confirmed by qRT-PCR)	Downregulated genes (confirmed by qRT-PCR)	Related biomarkers	Reference
Postprandial, randomized, crossover	20 healthy, elderly individuals	Three diets during 3 weeks (MedDiet, SFA-rich diet, low-fat and CHO-PUFA enriched diet	PBMCs	qRT-PCR	IκBa after MedDiet versus both other diets.	p65 subunit of NFkB, MCP-1, and MMP-9 after MedDiet versus SFA-diet.p65 subunit of NFkB and TNFα after MedDiet versus CHO-PUFA diet	↑ plasma MCP1 after SFA diet. ↑ plasma IL6 and ↓ plasma TNFα after all diets.	Camargo et al. [26]
Postprandial, randomized, crossover	20 healthy, elderly individuals	Three isocaloric diets for 4 weeks (MedDiet+CoQ, MedDiet, and SFA diet) and three similar breakfasts at fasting state	PBMCs	qRT-PCR	–	NRF2, $p22^{phox}$, $p47^{phox}$ SOD1, SOD2, TRXR after MedDiet and MedDiet+CoQ versus SFA diet	↑ cytoplasmatic Nrf2 and Keap-1 proteins after MedDiet and MedDiet+CoQ	Yubero-Serrano et al. [27]
Postprandial, randomized, crossover	20 healthy, elderly individuals	Three isocaloric diets for 4 weeks (MedDiet+CoQ, MedDiet, and SFA diet) and three similar breakfasts at fasting state	PBMCs	qRT-PCR	IkBa after MedDiet and MedDiet+CoQ versus SFA diet	IL1b, JNK-1, p65, IKK-b, MMP9, sXBP1, BiP/Grp78 after MedDiet and MedDiet+CoQ versus SFA diet	–	Yubero-Serrano et al. [28]
Randomized, parallel intervention	25 women with MetS	Medstyle versus Medstyle+antioxidant rich beverage for 12 weeks	PBMCs	qRT-PCR	–	HMB-CoA versus baseline in both groups	↓ plasma insulin and↓ LDL-C versus baseline	Jones et al. [29]
Postprandial, randomized, crossover	Eight healthy men	Three meals with different FA composition (OO, butter, and walnut meal)	PBMCs	EMSA	NFkB transcription factor activation after butter and walnut meals, but not OO	–	↑ sICAM1 after butter meal	Bellido et al. [33]
Postprandial randomized, crossover	20 healthy men	Four diets (Western, MedDiet, CHO-rich, and n-3 diets) during 4 weeks and three breakfasts (butter, OO, and walnut-rich)	PBMCs	qRT-PCR	↑TNFa after butter breakfast, ↑ IL6 after butter, and OO breakfasts	–	–	Jimenez-Gomez et al. [34]

Table 1. Continued

Type of study	Participants	Intervention	Tissue	Measured by	Upregulated genes (confirmed by qRT-PCR)	Downregulated genes (confirmed by qRT-PCR)	Related biomarkers	Reference
Postprandial, linear intervention	Six healthy men	Single dose VOO consumption (50 mL)	PBMCs	Human Genome Survey Microarray (MA)Applied Biosystems	ADAM17, AKAP13, IL10, OGT, USP48 General pathways in MA[a)]: Metabolism, primary metabolism, regulation of biological process genes, etc.	IFNγ General pathways in MA[a)]: Biosynthesis, response to biotic stimulus, defense response, etc.	–	Konstantinidou et al. [35]
Postprandial linear intervention	11 healthy individuals (six men and five women)	Single dose VOO consumption (50 mL)	PBMCs	qRT-PCR	ADAM17, ADRB2, LIAS, PPARBP, and OGT at 6 h versus 0 h.CD36 at 1 h versus 0 h	OGT and ALOX5AP at 1 h versus 0 hALOX5AP at 1 h versus 0 h	↑ Insulin, glucose (1 h) ↑ LDLox and TBARS (6 h)	Konstantinidou et al. [37]
Postprandial, randomized, double-blinded, crossover trial	20 individuals with three MetS features	Two breakfasts based on OO with high and low polyphenols (60 g of white bread with 40 mL of oil)	PBMCS	Two-color microarray (Agilent)	–	JUN, PTGS2, EGR1, and IL1B after high-polyphenols OO versus low-polyphenols OO General pathways in MA Inflammatory-related disorder		Camargo et al. [38]
Linear, controlled	Ten healthy individuals (six men and four women)	VOO consumption (25 mL/day) for 3 weeks	PBMCs	Human Genome Survey Microarray (MA) Applied Biosystems	ADAM17, ALDH1A1, BIRC1, ERCC5, LIAS, OGT, PPARBP, TNFSF10, USP48, XRCC5	–	↓ Triglycerides	Khymenets et al. [22]
Randomized, double-blind, parallel intervention	81 healthy post-menopausal women	CLA-rich diet versus OO-rich diet for 16 weeks	Adipose tissue	qRT-PCR	TNFa in CLA-rich diet versus OO-rich	GLUT4, LPL, and LEP in CLA-rich diet versus OO-rich	↓ Body fat mass and ↑ Serum insulin in the CLA-rich diet	Raff et al. [41]

Table 1. Continued

Type of study	Participants	Intervention	Tissue	Measured by	Upregulated genes (confirmed by qRT-PCR)	Downregulated genes (confirmed by qRT-PCR)	Related biomarkers	Reference
Parallel controlled-feeding trial	20 individuals at risk of metabolic syndrome	SFA-rich diet versus MUFA-rich diet for 8 weeks	Plasma and adipose tissue	Whole-Genome GeneChip Microarray (MA)(Affymetrix)	CCL5/RANTES, CD14 and RAC2 after SFA rich. General pathways in MA[a]: MUFA diet: p38 MAPK signaling, phospholipid degradation, eicosanoid signaling, pyruvate metabolism	ADIPOQ after SFA and CD163 after MUFA General pathways in MA[a]: MUFA diet: hepatic fibrosis, glycolysis, complement system, fatty acid biosynthesis, biosynthesis of steroids, taurine metabolism	↓ Serum total cholesterol and LDL-C, and ↑ in plasma oleic acid after MUFA diet	Van Dijk et al. [42]
Randomized, crossover, controlled trial	18 healthy men	25 mL OO with a low polyphenol content versus a high polyphenol content for 3 weeks	PBMCs	qRT-PCR	None	CD40L, IL23A, ADRB2, OLR1, IL8RA, IL7R, IFNγ, and MCP1 after high polyphenol OO	↓ LDL-C ↓ total cholesterol ↓ MCP1 after polyphenol-rich OO	Castañer et al. [43]
Randomized, parallel, double-blind	49 high CVD—risk patients	TMD + VOO versus TMD + nuts versus low fat diet for 12 weeks	PBMCs	qRT-PCR	LRP1 and MCP1 in TMD + nuts and control group	COX2, LRP1, MCP1 in TMD+VOO group	↓ SBP, glucose, total cholesterol, and LDL-C after TMD VOO	Llorente-Cortes et al. [46]
Randomized, parallel, double-blind	90 healthy individuals (26 men and 64 women)	TMD + VOO versus TMD + WOO (25 mL/day) versus control for 12 weeks	PBMCs	qRT-PCR	None	ADRB2, ARHGAP15, IFNγ, and IL7R in TMD+VOOPOLK in TMD	↓ In total cholesterol, HDL-C, LDL-C, IFNγ, sP-selectin, F_{2a}-isoprostanes after TMD+VOO	Konstantinidou et al. [47]

a) Microarray results only.
↓ decrease; ↑ increase.
ADAM17, ADAM metallopeptidase domain 17; ADRB2, adrenoceptor beta 2; ALDH1A1, aldehyde dehydrogenase 1 family, member A1; ALOX5AP, arachidonate 5-lipoxygenase-activating protein; ARHGAP15, rho GTPase activating protein 15; BiP/Grp78, glucose-regulated protein, 78kDa; BIRC1, NLR family, apoptosis inhibitory protein; CLA, conjugated linoleic acids; CCL5/RANTES, regulated on activation normal T cell expressed and secreted/chemokine CC motif ligand 5; CD14, CD14 molecule; CD36, CD36 molecule (thrombospondin receptor); CD40/CD40L, CD40 antigen ligand; CD163, CD163 molecule; CoQ, coenzyme Q10; COX2, cyclooxigenase-2; ERCC5, DNA excision repair protein; EVOO, extra virgin olive oil; EMSA, electrophoretic mobility shift assay; FASN, fatty acid synthase; GLUT4, glucose transporter 4; JNK1, Jun N-terminal kinase 1 or mitogen-activated protein kinase 8; IKBa, NF-kappa-B inhibitor alpha; IKKb, inhibitor of nuclear factor kappa-B kinase subunit beta; IL6, interleukin 6; IL7R, interleukin 7 receptor; IL10, interleukin 10; IFNγ, interferon gamma; LDL-C, low-density lipoprotein cholesterol; LDL-ox, oxidized LDL; LEP, leptin; LIAS, lipoic acid synthetase; LPL, lipoprotein lipase; LRP1, low-density lipoprotein receptor-related protein; MCP1, monocyte chemoattractant protein 1; MedDiet, Mediterranean diet; Medstyle, Mediterranean-style low-glycemic load diet; MetS, metabolic syndrome; MMP9, matrix metallopeptidase 9; mRNA, messenger RNA; NFκB, nuclear factor NF-kappa-B; NRF2, nuclear factor (erythroid-derived 2)-like 2; OGT, O-linked *N*-acetylglucosamine (GlcNAc) transferase; OO, olive oil; PBMCs, peripheral blood mononuclear cells; PPARBP, peroxisome proliferator-activated receptor-binding proteína; POLK, polymerase κ; p22phoxand p47phox, NADPH oxidase subunits; RAC2, ras-related C3 botulinum toxin substrate 2; SBP, systolic blood pressure; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; sXBP1, X-box binding protein 1; TG, triglycerides; TMD, traditional Mediterranean diet; WOO, washed olive oil; TNFα, tumor necrosis factor α; TNFSF10, tumor necrosis factor (ligand) superfamily, member 10; TRXR, thioredoxin reductase; USP48, ubiquitin-specific peptidase 48; VOO, virgin olive oil; XRCC5, x-ray repair complementing defective repair.

The binding between a phenolic compound and the target protein is determined by their structural relationship. This implies that different phenolic compounds and/or phytochemicals have different target proteins. The phytochemical structure plays a predominant role not only in the direct scavenging of free radicals, but also in the molecular mechanisms involved. Up to now, the human trials that have been performed tested the nutrigenomic effects of (i) OO versus other type of oils, (ii) OOs with differences in their polyphenols content, and (iii) OOs with differences in their fatty acid content (Table 1).

2.2.1 Single dose postprandial studies

In 2004, Bellido et al. examined the postprandial activation of the nuclear transcription factor NFκB in human PBMCs, after breakfasts rich in butter, walnuts, or OO [33]. Eight healthy men participated in this postprandial study after having followed a 4-week baseline diet. The breakfasts had a similar fat-load consisting of 1 g fat/kg body weight (65% fat). The results showed that only butter- and walnut-enriched meals elicited the NFκB postprandial activation in PBMCs of the healthy volunteers. Also, in a randomized, crossover study with 20 healthy men who followed three diets (MedDiet, Western, and CHO-rich) during 4 weeks, a butter breakfast elicited a higher increase in *TNF*α mRNA expression when compared to OO or walnut breakfasts. However, there was a higher postprandial *IL6* expression after the butter and OO breakfasts than with the walnut one [34].

In our first exploratory report, we assessed human in vivo gene expression changes after ingestion of a single 50 mL dose of VOO by analyzing microarray data [35]. The aim was to assess gene expression changes in PBMCs of healthy volunteers 6 h after the VOO dose. This time point was selected because it reflects the end of the postprandial state [36]. The highest upregulation was observed in genes related to metabolism, cellular processes, and cancer. The highest downregulation appeared in genes related to environmental information processing pathways. Microarray results were verified by qRT-PCR in all five upregulated genes (*ADAM17*, *IL10*, *OGT*, *USP48*, and *AKAP13*). For the downregulated ones, full concordance was achieved only in the case of *IFN*γ gene. This acute 50 mL VOO dose also promoted in vivo time-course changes, as measured by qRT-PCR, in the expression of genes (*ADAM17*, *ADRB2*, *ALOX5AP*, *CD36*, *LIAS*, *OGT*, *PPARBP*) related to insulin resistance, oxidative stress, and inflammation in healthy volunteers [37]. Some genes, *OGT* and *ALO5AP* followed an inversed quadratic trend with a decrease at 1 h postprandial followed by an increase at 6 h, whereas in the case of *CD36* the quadratic trend was a direct one. Other genes, *ADAM17*, *ADRB2*, *LIAS*, and *PPARBP* followed an increasing linear trend through the postprandial phase. *ALOX5AP* and *OGT* gene expression changes were inversely correlated with insulin and glucose levels at 1 h postprandial. *ADAM17* and *ADRB2* gene expression was inversely correlated with plasma oxidized LDL concentrations at 6 h postprandial [37].

Camargo et al. [38] have reported that a breakfast based on VOO, high in polyphenols (398 ppm), was able to postprandially repress the expression of proinflammatory genes when compared with a common OO based breakfast (low in polyphenols, 70 ppm) (Table 1). Twenty adults (9 men, 11 women), fulfilling at least three criteria for metabolic syndrome [39], participated in this randomized crossover trial. Two-color microarrays (Agilent) were performed showing 19 upregulated and 79 downregulated genes, linked to obesity, dyslipemia, and type 2 diabetes mellitus, after the intake of OO high in polyphenols. qRT-PCR verification showed a decreased expression of *IL1B*, *PTGS2* (*COX2*), *JUN*, and *EGR1* genes after high-phenol OO breakfast when compared to low-phenol OO one.

2.2.2 Sustained consumption

Ten healthy individuals, six men and four women, participated in a linear study with an intervention period of 3 weeks with a daily VOO consumption of (25 mL/day), a common intake in the MedDiet. In PBMCs, we reported an upregulation in the expression of genes associated with atherosclerosis-related processes such as *ADAM17*, *ALDH1A1*, *BIRC1*, *ERCC5*, *LIAS*, *OGT*, *PPARBP*, *TNFSF10*, *USP48*, and *XRCC5* [22].

Concerning adipose tissue, the adipose tissue-derived macrophages are the source of inflammatory pathways and obesity development, a well-known CVD risk factor. Obesity development is associated with the progressive infiltration of monocytes and macrophages into adipose tissue [40]. The effect of diets supplemented with conjugated linoleic acids (CLAs) versus OO, after a 16-week consumption, on adipocyte gene expression changes were examined in a randomized, parallel, double-blind trial [41]. In this study, 81 healthy postmenopausal women participated. The mRNA expression of the glucose transporter4 (*GLUT4*), leptin **(***LEP***)**, and lipoprotein lipase (*LPL*) was lower, and that of *TNF*α was higher, in the group following 16-week consumption of CLA compared to the OO group. Concerning systemic biomarkers, the authors observed an increase in the serum insulin levels after the CLA diet only in women with high baseline waist circumference. Also, they saw a decrease in the total fat mass after CLA consumption [41].

A specific effect of the MUFA-rich diet, mainly in the form of refined OO, versus SFA-rich diets on gene expression has also been reported [42]. In this parallel, controlled-feeding trial, 20 abdominally overweight subjects participated during 8 weeks, and adipose tissue samples were collected for gene expression analyses. The MUFA diet led to a more anti-inflammatory gene expression profile: upregulation of phospholipid metabolism, degradation, and eicosanoid signaling pathways and downregulation of the complement system, fatty acid, and steroids biosynthesis pathways among others.

Consumption of the SFA diet resulted in a proinflammatory "obesity-linked" profile: upregulation of immune function and inflammation-related processes and downregulation of amino acid metabolism and fatty acid metabolism. Moreover, MUFA intake promoted a decrease in serum LDL cholesterol and an increase in plasma and adipose tissue oleic acid content. Microarray results were validated by q-PCR analysis in a set of genes involved in inflammatory processes. The *ADIPOQ* gene was downregulated and *CCL5*/*RANTES*, *CD14*, and *RAC2* genes were upregulated both in MA and qPCR results, after SFA-rich diet. After MUFA-rich diet the *CD163* gene was downregulated. Concerning the other four genes tested (*C1QB*, *CTSS*, *ITGB2*, *PPAR*γ), qPCR changes did not reach statistical significance.

The first study proposing a possible molecular scheme of action after polyphenol-rich OO consumption in a human intervention study was recently published by Castañer et al. [43]. In this substudy of the EUROLIVE study [12], healthy men followed a 3-week crossover intervention with a daily 25 mL consumption of raw OO with high- versus low-content in polyphenols. Results showed a decrease in the expression of proatherogenic genes (*CD40L*, *IL23A*, *ADRB2*, *OLR1*, *IL8RA*, and *IL7R*) specifically after consumption of OO high in polyphenols when compared with the OO low in polyphenols. The decrease in these genes was concomitant with the decreasing trend of interlinked ones such as *VEGF*, *ICAM1*, and *MCP1*.

In this study [43], we proposed an integrated scheme for the in vivo downregulation of the CD40/CD40L system and its downstream products promoted by OO polyphenol consumption. CD40 and sCD40L belong to the tumor necrosis factor superfamily and they are molecules with a dual prothrombotic and proinflammatory role [44]. The CD40L pair triggers inflammatory signals in cells of the vascular wall, representing a major pathogenetic pathway of atherosclerosis [45]. Systemic LDL oxidation, total cholesterol, and plasma MCP1 decreased after the high-polyphenols intervention compared with after the low-polyphenols intervention. The reduction in LDL oxidation and the increase in antioxidant polyphenols, promoted by the regular dietary intake of polyphenol-rich OO, were associated with a downregulation in the expression of genes related with the CD40/CD40L pathway.

2.3 Combined transcriptomic effect of the MedDiet, OO, and its polyphenols

Data from a subsample of the PREDIMED study [15] have shown that a 3-month intervention with VOO-enriched TMD (i) prevented the increase in cyclooxigenase-2 (*COX2*) and LDL receptor-related protein (*LRP1*) genes, and (ii) reduced the expression of monocyte chemoattractant protein (*MCP1*) gene, compared with a TMD enriched with nuts or with a low-fat diet [46]. *COX2* and *MCP1* genes are involved in inflammation, whereas LRP1 is involved in foam cell formation. In this study 49 asymptomatic high cardiovascular-risk patients participated, and gene expression changes were assessed in PBMCs. A decrease in systolic blood pressure, plasma glucose, total, and LDL cholesterol was also reported in the TMD group enriched with VOO versus baseline values. In the same group, changes in systolic and diastolic blood pressure positively correlated with changes in *LRP1* expression. In the TMD group enriched with nuts a decrease in systolic and diastolic blood pressure and in glucose levels compared with baseline was also observed.

We have reported an in vivo nutrigenomic effect of OO polyphenols in humans within the frame of the TMD [47]. In a parallel, controlled clinical, intervention trial 90 healthy volunteers were randomized and followed three intervention dietary patterns: (i) TMD with VOO, rich in polyphenols (328 mg/kg), (ii) TMD with washed VOO, low in polyphenols (55 mg/kg), and (iii) habitual diet (control group), during a 3-month period. TMD consumption (both groups 1 and 2) decreased plasma oxidative and inflammatory status and the expression of genes related with inflammation (*IFN*γ, *ARHGAP15*, and *IL7R*), oxidative stress (*ADRB2*), and DNA damage (*POLK*) in PBMCS. To examine the contribution of the polyphenols present in OO to the gene expression decrease, the two TMDs were assessed separately. Only after TMD+VOO was a significant decrease versus the control group observed in all genes with the exception of *POLK*. Changes in gene expression after TMD were concomitant with decreases in lipid oxidative damage and systemic inflammation markers.

2.4 Comments

There were extensive differences among the studies revised, such as the lack of homogeneity of the study designs, the population involved, the measurement of biomarkers of compliance, the control of possible confounder variables (e.g. physical activity), and the end-point variables (i.e. gene expression), among others. In spite of this, it appears that there is a modulatory effect, toward a protective mode, of the MedDiet, its main fat component OO, and its polyphenols, on genes related to chronic degenerative diseases, particularly atherosclerotic processes, such as oxidation and inflammation. The OOs used in the studies cited here were in all cases natural, not-enriched, OOs. The evidence that phenolic compounds present in the OO were responsible for the transcriptomic effect is provided from randomized, controlled, human studies in which similar OOs, but with differences in their phenolic content have been tested in the trials. The statement that the OO or its polyphenols are responsible for the transcriptomic effect within the frame of the MedDiet came from randomized, controlled, human studies in which the MedDiets compared were or were not rich in OO, or had two similar OOs but with differences in their phenolic content. To the best of our knowledge, this is the first review on the transcriptomic effects of the MedDiet and OO consumption, in humans, performed on the basis of evidence-based medicine.

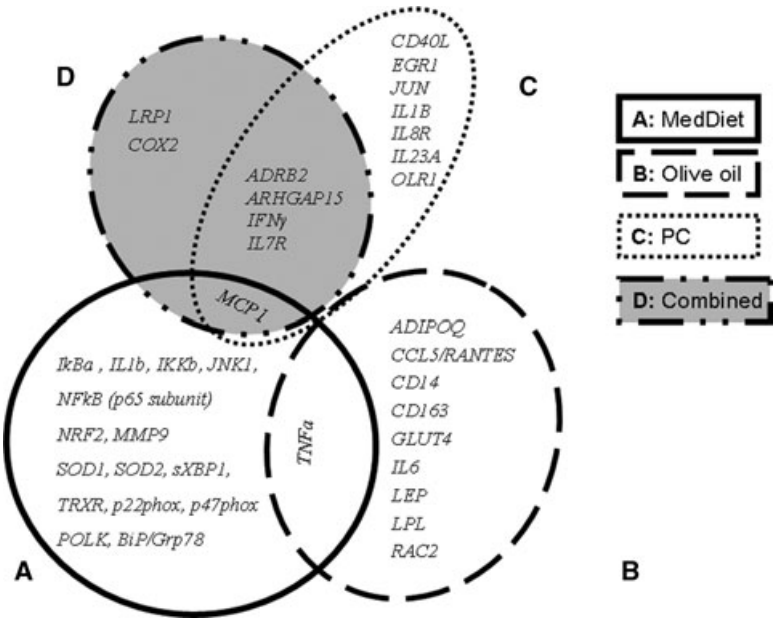

Figure 1. Group of genes differentially expressed, as verified by qRT-PCR, in transcriptomic studies in humans after (A) MedDiet intervention, (B) olive oil (OO) intervention, (C) OO phenolic compounds intervention, and (D) MedDiet and OO combined intervention.

Figure 1 shows the genes, verified by qRT-PCR, which were modulated by the MedDiet, OO, and its polyphenols. Data from exploratory studies in which no control group was present [22, 29, 35, 37] are excluded. In Fig. 1 we established four groups of genes, appearing in the results of the available human transcriptomic studies after consumption of: (i) MedDiet, (ii) OO, (iii) OO polyphenols, and (iv) combined MedDiet and OO. Some genes were modulated in two or three of these study categories. This overlapping among categories points out that some genes, such as TNFα and MCP1, are modulated by OO and OO polyphenols, respectively, within and out of the context of the MedDiet [26, 34, 41, 46]. Monocyte chemoattractant protein-1 (*MCP1*) (also known as CCL2, chemokine C-C motif ligand 2) is a crucial chemokine responsible for the recruitment of monocytes to inflammatory lesions in the vasculature [48]. It plays a fundamental role in the recruitment of monocytes to sites of injury and infection, and has been shown to increase in chronic inflammation, such as rheumatoid arthritis or lupus [49]. *MCP1* was downregulated after 3 weeks of adherence to the MedDiet, compared to the SFA-diet [26]. When the MedDiet was supplemented with VOO, and after a 12-week consumption, the overexpression of *MCP1* was prevented [46], suggesting a role of this gene in the anti-inflammatory properties of VOO. The *TNF*α gene acts as an activator of a cascade of cytokine production [50] and is also considered to be a crucial proinflammatory cytokine. The *TNF*α gene appeared upregulated after consumption of a butter-rich breakfast [34], and a CLA-rich diet [41], but not after OO-rich breakfast or diet. This fact points out a protective effect of OO on inflammatory processes.

Furthermore, interferon gamma (*IFN*γ) and interleukin-7 receptor (*IL7R*) appear to be downregulated in more than one study [43, 47]. *IFN*γ and *IL7R* are key proinflammatory cytokines, which were downregulated after sustained high polyphenols OO consumption within and out of the MedDiet [42, 47]. *IFN*γ is considered to be a key inflammatory mediator for inducing *IL6*, a prime regulator of CRP synthesis in the liver [47]. The protein encoded by the *IL7R* gene is a receptor for IL7, which has been related to inflammatory processes and shown to enhance the expression of chemokines in PBMNCs [51]. OO polyphenols seem to play a crucial role in the downregulation of *IFN*γ and *IL7R* genes when consumed within the frame of the MedDiet. In one study [47], systemic plasma IFNγ levels were significantly decreased after 3 months of high polyphenols OO consumption. This decrease also correlated with the downregulation of the *IFN*γ gene.

ADRB2 gene also seems to have a crucial role in explaining the health benefits of OO and the MedDiet. *ADRB2* gene was downregulated after consumption of polyphenols-rich OO, within and out of the frame of the MedDiet [43, 47]. Although the role of β-adrenergic receptors in CVD is not yet clear, overexpression of β-adrenergic receptors has been implicated in the progression of the disease. A recent genome wide expression study has shown an upregulation of *ADRB2* in patients with premature familial coronary artery disease [52]. This upregulation was reduced when the patients were treated with statins and aspirin. In our previous functional studies after VOO ingestion, the increase in postprandial *ADRB2* expression was inversely related to LDL oxidation. All these data suggest a protective role of the *ADRB2* downregulation in oxidative stress [37].

Transcriptomic changes after MedDiet, OO, and its polyphenols consumption were included within pathways such as oxidoreductase activity (*JUN*), hydroxymethylglutaryl-CoA reductase activity (*HMB-CoA*), adipocytokine receptor signaling pathway (*ADIPOQ,GLUT4, NFκB, TNF*α), *VEGF* signaling pathway (*COX2*), hematopoietic cell lineage (*CD14*), and cytokine–cytokine receptor interaction (*CCL5,*

LEP, IL6, IL8R, IL7R, IL1B, TNFα, IFNγ). These pathways are where most of the candidate genes for predicting coronary artery disease are included [53]. *MMP9*, a metalloproteinase downregulated by the MedDiet, has been shown to be expressed in mitral and aortic valves of patients with endocartiditis and degenerative valvular diseases [54]. Increased expression of leptin, a gene downregulated by OO consumption in PBMC, in resident macrophages characterizes atherosclerotic plaque rupture [55]. The CD40/CD40L system is considered to be proatherogenic and prothrombotic and links inflammation with atherothrombosis [56]. The *CD40* expression has been shown to be downregulated by OO polyphenols. A key downstream product of the CD40/CD40L cascade is *MCP1*, which is a potent regulator of leukocyte trafficking. Data show that *MCP1* expression was downregulated by MedDiet, OO, and OO polyphenol consumption. This cytokine is involved in the pathogenesis of diseases characterized by monocytic infiltrates, such as vascular diseases [57]. All the above mentioned reinforces the nutrigenomic effect as one of the mechanisms by which the MedDiet and its main component, OO, could exert a protective effect against atherosclerosis and CVD development.

The small number of participants, the brief duration of the interventions, and the lack of replication in some of the results obtained remain the main limitations of the studies conducted up to now. Confirmation of the above results in different and larger populations is warranted. Whether additional or different effects would have been observed over longer intervention periods is unknown. Longer interventions, however, could impair the compliance of the participants. During nutritional intervention studies the compliance of volunteers plays a crucial role. Data from the predictive value of gene expression changes, however, is mainly provided by case-control studies, instead of cohort ones that can provide the first level of evidence [18]. Due to this, the clinical significance of the changes observed after MedDiet, OO, and its phenolic compounds must be considered as preliminary data.

Specific nutritional biomarkers should be also standardized and used to ensure that the observed effect is attributed to the administered food or food component. For example, in the case of OO consumption, tyrosol and hydroxytyrosol levels in urine are used as validated biomarkers to assess participant compliance concerning the type of OO ingested [58, 59].

During human intervention studies with the MedDiet pattern there is always the inability to assess potential interactions between the OO and other diet components that might affect the generalization of the results. For this reason, an adequate control group should be chosen in each case. The effects of naturally consumed food components are always subtle and must be considered within the context of chronic exposure. Changes in gene expression were modest as expected in real-life conditions where a nonpharmacological response exists. Up to now, the majority of human nutrigenomic studies use PBMCs as a feasible matrix for gene expression assessment. However, the nutrigenomic response can be tissue specific, which limits the extrapolation of the results. The need for gene expression measurements in human tissues, other than PBMCs, such as adipose tissue is a future aim.

A primordial challenge of nutrigenomic studies in humans should also be the correlation of gene expression changes with systemic results. Some nonconcordance between the transcriptomic and proteomic data could be due to different time-serial response, half-life of proteins, limitations of methodologies, and/or different cellular lines involved in the gene expression. Linking up results from the different "omics" techniques and the classical, biomarkers approach will help us achieve a solid, holistic view of how diet can affect our genes. Moreover, the use of proper atherosclerosis animal models to elucidate the potential mechanisms of the interesting transcriptomic effect of the MedDiet is highly recommended to clarify the exact in vivo mechanisms. From a genomic point of view, MedDiet could be a dietary pattern closer to that of our ancestors and thus more compatible with our genes and the entire machinery depending on them.

3 Summary of findings

There are extensive differences among the studies assessing the human transcriptomic response after the MedDiet and OO consumption, such as the lack of homogeneity of the study designs, the differences in their end-point variables (i.e. gene expression), and the sample population. Results from our critical review show that the benefits associated with OO and MedDiet consumption on human health could be mediated through changes in the expression of genes related to chronic degenerative diseases, particularly inflammation and oxidative stress (i.e. *IFNγ, IL7R, ADRB2, MCP1, TNFα*). Despite the observed heterogeneity of the studies, it is accepted that OO, the main source of fat in the MedDiet, is more than a MUFA-rich source and its polyphenols could account for greater nutrigenomic, protective effects. The need to evaluate gene expression changes in human tissues, other than PBMCs, and verification of the above results in larger populations is also warranted.

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