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REVIEW

Gene expression of peripheral blood mononuclear cells as a tool in dietary intervention studies: What do we know so far?

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Peripheral blood mononuclear cells (PBMCs) generally refer to monocytes and lymphocytes, representing cells of the innate and adaptive immune systems. PBMCs are a promising target tissue in the field of nutrigenomics because they seem to reflect the effects of dietary modifications at the level of gene expression. In this review, we describe and discuss the scientific literature concerning the use of gene expression at the mRNA level measured from PBMCs in dietary interventions studies conducted in humans. A search of literature was undertaken using PubMed (last assessed November 24, 2011) and 20 articles were selected for discussion. Currently, results from these studies showed that PBMCs seem to reflect liver environment and complement adipose tissue findings in transcriptomics. PBMC gene expression after dietary intervention studies can be used for studying the response of certain genes related to fatty acid and cholesterol metabolism, and to explore the response of dietary interventions in relation to inflammation. However, PBMC transcriptomics from dietary intervention studies have not resulted yet in clear confirmation of candidate genes related to disease risk. Use of microarray technology in larger well-designed dietary intervention studies is still needed for exploring PBMC potential in the field of nutrigenomics.

Keywords:
Diet / Inflammation / Intervention / mRNA expression / Peripheral blood mononuclear cells

1 Introduction

1.1 Immune function: General remarks and the peripheral blood mononuclear cells (PBMCs)

The main function of the immune system is to prevent or limit infections by microorganisms such as bacteria, viruses, fungi, and parasites. Immune responses are mediated by leukocytes (white blood cells), which derive from precursors in the bone marrow and then migrate to guard peripheral tissues [1]. Cells of the innate immune system, such as monocytes, macrophages, and neutrophils, provide the first line of defense against bacterial infections. [1]. Innate immune responses involve secretion of cytokines from the innate immune cells, which result in inflammation and further activation of the adaptive immune system [1]. For example,

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Abbreviations: AIDM, antiinflammatory diet mix; **CCL**, chemokine C-C motif ligand; **CPT1A**, carnitine palmitoyltransferase 1A; **CPT1B**, carnitine palmitoyltransferase 1B; **CVD**, cardiovascular disease; **DHA**, docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **FA**, fatty acid; **HMGCR**, 3-hydroxy-3-methyl-glutaryl-CoA reductase; **hsCRP**, high-sensitivity C-reactive protein; **IL17R**, IL17 receptor; **IR**, insulin resistance; **LRP**, LDL receptor protein; **NAMPT**, nicotinamide phosphoribosyltransferase; **NFκB**, nuclear factor-kappa-B; **PPAR**, peroxisome proliferator-activated receptor; **PBMCs**, peripheral blood mononuclear cells; **PDGF**, platelet-derived growth factor; **qRT-PCR**, quantitative RT-PCR; **PPC**, low-dose PUFA, polyphenols and L-carnitine supplementation; **S_I**, insulin sensitivity; **T2DM**, type 2 diabetes mellitus; **TLR**, toll-like receptor; **TNF**, tumor necrosis factor; **VOO**, virgin olive oil

lymphocytes are cells of the adaptive immune system, which recognize specific pathogens and act by protecting against recurrent infections. Lymphocytes are the largest cell population covered by the more general term PBMCs, which also include monocytes [1].

1.2 Challenges using mRNA gene expression in nutrigenomic studies

The methodologies used to study the messenger RNA (mRNA) expression of genes can vary from single gene expression methods to whole-genome expression profiles (transcriptomics) and pathway analyses. The methods mostly used to study the expression of single genes have been the competitive RT-PCR [2, 3], and more recently, quantitative RT-PCR (qRT-PCR) [4]. The development of high-throughput methods has opened a new avenue in the gene expression studies [5]. Genome-wide gene expression, e.g. transcriptomics research, has already identified novel genes and pathways that have not been earlier linked to certain treatments or conditions.

The term nutrigenomics was created to describe how nutrition affects gene and protein functions at genomic, transcriptional, proteomic, and metabolic levels [6]. Nutrients can affect the transcriptional level of genes by influencing transcriptional control and acting on specific transcriptional factors [7, 8]. For example, nuclear peroxisome proliferator-activated receptors (PPARs) serve as important fatty acid (FA) sensors in the cells. Other examples of transcription factors that can be affected by dietary components such as flavonoids, vitamin E, and cholesterol are nuclear factor-kappa-B (NFκB), the pregnane X receptor, and sterol-responsive-element binding proteins, respectively [8].

Prevention of chronic diseases such as cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) is a major focus of research in the nutrition field. Data have shown that these conditions can be prevented by lifestyle changes, including changes toward a healthier diet [9, 10]. Development of biomarkers of food intake is of great interest because they can be used as indicators of metabolic or physiological changes that may increase or decrease the risk of a specific chronic disease. These biomarkers should reflect or be associated with these conditions and phenotypes. Classical biomarkers such as blood lipids, plasma glucose, and measurements of insulin resistance (IR) are widely used for addressing the effect of dietary intervention on risk factors for T2DM and CVD. The use of transcriptomics can help to provide more information about eventual biomarkers of certain diseases or physiological changes related to the pathogenesis of the disease. Moreover, early biomarkers or transcriptomic profiles ("signatures") could emerge from this molecular approach, which could ultimate give us more time to intervene in the prevention of a disease that may develop in the future.

In order to study the role of food and nutrients in target tissues and to understand the molecular mechanisms behind the beneficial effects of diet on health, usually liver, adipose, and muscle tissues are of prime interest. However, often in the studies with healthy human volunteers tissue availability sets limitations. The adipose and muscle tissues are rather accessible, but the amount of the samples might not be sufficient for performing gene expression profiling, confirmation by RT-PCR and protein analyses. On the other hand, liver samples are usually impossible to obtain from healthy volunteers. Because PBMCs seem to reflect hepatic regulation of cholesterol metabolism [11] and can migrate through the blood circulation and infiltrate various tissues such as the endothelium and adipose tissue [12], PBMC gene expression might reflect metabolic and immune responses of adipocytes or hepatocytes. Since PBMCs can also reflect the responses of dietary modifications and drugs at the level of gene expression [13, 14], they have also been a subject of great interest in clinical and intervention studies for transcriptomics profiling. Moreover, PBMCs are convenient because they can be easily and repeatedly collected in sufficient quantities, in contrast to adipose, muscle, and liver tissues.

Immune cells such as lymphocytes and monocytes (PBMCs) seem to play a crucial role in the development of the atherosclerotic lesion [15] because of their possible roles in the development of inflammation (cytokine production), endothelial dysfunction (endothelium adhesion molecule production), and CVD [16]. Mechanisms contributing to IR, impaired β-cell function and endothelial dysfunction also include inflammation [16, 17]. Obesity and IR seem to activate immune cells and increase expression of many cytokines [18, 19]. Moreover, cholesterol metabolism can also participate in the activation of inflammation at the artery wall site [20].

PBMCs have been used for exploring gene expression in various diseases and to predict clinical outcome [21–23], and also as a model for studying the expression of genes related to inflammation [24–27]. Moreover, PBMCs are also a target for insulin action [28]. Of note, these immune cells are not only exposed to IR- and CVD-related tissues such as adipose, liver, and the endothelium, but also crosstalk with them [29]. Global gene expression profile of skeletal muscle seems also to be reflected in the gene expression levels of PBMCs [30]. Therefore, the use of changes in PBMC gene expression in dietary intervention studies as a model system could also suit the aim of understanding the underlying mechanisms behind the role of diet and nutrients in atherosclerosis or IR leading to CVD and T2DM.

One of the aims of nutrigenomics is to identify the genes that influence diet-related diseases such as T2DM and CVD. This could also ultimately lead to the identification of genotypes that could especially benefit from specific dietary interventions. There is recent evidence for an extensive genetic component underlying gene expression traits in PBMCs [31]. The transcripts of genes measured from PBMCs seem to be heritable [32, 33]. In this regard, the information derived from transcriptomic studies in dietary intervention studies can also be used for identifying candidate genes for various diseases. For example, we have found that the tenomodulin (TNMD)

gene, which was downregulated after moderate weight loss [34], was associated with inflammatory factors and risk of T2DM in the Finnish Diabetes Prevention Study [35, 36]. Therefore, it is reasonable to expect that new insights could emerge from future PBMCs transcriptomics in terms of new candidate genes related to T2DM and CVDs.

In the present review, we describe and discuss the scientific literature concerning the use of gene expression at mRNA level measured in PBMCs in dietary interventions studies conducted in humans. We will mainly focus on controlled trials with a minimum length of 4 weeks.

2 Methodology for the literature searching

A search of medical literature was undertaken using PubMed (last assessed, November 24, 2011) with the following keywords in combinations of three: "gene expression," "mRNA expression," "transcriptome," OR "nutrigenomics" AND "diet," "dietary intervention," "weight loss," "weight reduction" OR "supplementation," AND "PBMC" OR "mononuclear cells." Concerning "supplementation," only human studies dealing with dietary intervention or supplementation of *n*-3 FAs were selected. After excluding six manuscripts dealing only with postprandial effects of a dietary or meal intervention, one manuscript dealing with supplementation with FAs using parenteral nutrition, one manuscript dealing with dietary intervention but using mRNA expression in white blood cells as a marker of DNA damage and another manuscript dealing with dietary intervention but using mRNA expression in PBMCs for its validation against skeletal muscle tissue in a cross-sectional design, 20 studies were left. In Table 1, the most relevant studies based on their design (controlled studies) and on their length (minimum of 4 weeks) are described. The other nine articles, which were from noncontrolled, single-arm studies or studies with a shorter duration, are discussed through in the text.

3 Dietary modifications and mRNA expression response associated with dietary FA manipulation

With the aim of better understanding the underlying mechanisms of the beneficial effect of the *n*-3 FAs on inflammation and atherosclerosis, two studies conducted by the same research group assessed the effect of *n*-3 FAs supplementation on PBMC gene expression response. In both of these studies, which are presented below, the authors selected the candidate genes according to what was currently assumed to play an important role in the pathogenesis of human atherosclerosis [37, 38].

The first dietary intervention study was published in 1993 [37] (Table 1). The intervention involved supplementation with *n*-3 FAs based on the grounds of the so far documented effects of these FAs on CVD at that time. The authors demonstrated for the first time that dietary *n*-3 FAs could influence PBMC mRNA expression in vivo. It was observed that *n*-3 FAs reduced the mRNA levels of platelet-derived growth factor (PDGF), which could result in reduced circulating levels of PDGF. In the subsequent study [38] (Table 1) with a similar study population and design, in addition to PDGF-α polypeptide (PDGFA) and PDGF-β polypeptide (PDFGB), supplementation with *n*-3 FAs also downregulated the mRNA expression of chemokine C-C motif ligand 2 (CCL2), the gene encoding for chemokine monocyte chemotactic protein 1 (MCP-1), known to play a role in CVD [39]. Similar results were observed in the same study after supplementing freshly isolated PBMCs that were unstimulated and stimulated for 20 h by adherence with *n*-3 FAs, reinforcing the in vivo findings [38].

With the aim to explore the inflammatory response at the mRNA level and clarify the mechanisms of the beneficial effect of *n*-3 FAs (fatty fish intake) or lean fish intake on the serum FA profile and plasma metabolic compounds related to IR, our research group found that the change in FA composition resulting from the fatty fish diet intervention was associated with an antiinflammatory response at the mRNA level in PBMCs [40] (Table 1). In previous studies, this antiinflammatory response has been associated with the improvement of insulin sensitivity (S_I) [41]. However, no significant differences between the experimental diets and the control group in the mRNA expression response of the candidate genes selected, which were all related either to inflammation or endothelial function, were observed (Table 1).

In another study, using fish oil supplementation as source of very long-chain FAs instead of fatty fish, Bouwens et al. showed that this supplementation can alter gene expression at mRNA levels in PBMCs to a more antiinflammatory and antiatherogenic direction [42]. The authors aimed to study the effects of long-term EPA + DHA supplementation on whole-genome gene expression profile to elucidate the mode of action of these FAs in human immune cells (Table 1). Although circulating high-sensitivity C-reactive protein (hsCRP) levels were not reduced with the intervention, interpretation of data derived from the transcriptomic analyses seemed to have brought new information to the underlying mechanisms of the beneficial effect of *n*-3 FAs on inflammation and processes involved in atherosclerosis and CVDs. For example, genes known to play a role in the atherosclerotic plaque formation and plaque stability were downregulated after EPA + DHA supplementation. Improvement of the preatherosclerotic condition in the subjects was also speculated based on the transcriptomic findings. Using qRT-PCR to confirm the results, the authors observed a dose–response reaction in the expression of genes of interest reflecting most of the pathways that were shown to be modulated by the fish oil supplementation (Table 1). Of note, for qRT-PCR measurements, 111

Table 1. Dietary intervention studies aiming at measuring mRNA expression of genes in peripheral blood mononuclear cells (PBMCs) listed in a chronological order

Study	Aim/purpose[a)]	Study design	Intervention(s)	Population[b)]	Intervention duration	Diet compliance	Methodology for the mRNA expression studies
Kaminski et al., 1993 [37]	To study if mRNA expression of atherosclerosis-related genes can be modulated by *n*-3 FAs.	RCT	Western diet + 7 g/day fish oil vs. Western diet (control)	Fourteen apparently lean and healthy young males (seven participants in each study group)	Six weeks	FA composition of phospholipids in PBMCs	3*n*-PCR
Baumann et al., 1999 [38]	To study if other FA classes than *n*-3 FAs modulate mRNA expression of PDGF, and also IL10, HBEGF, and CCL2.	Investigator-blinded, RCT	Individuals' usual diet plus either *n*-3, *n*-6, or *n*-9 FAs supplements (capsules) or usual diet (control)	Twenty-eight young healthy males (seven participants in each study group)	Twelve weeks	Returned capsules and plasma FA composition in phospholipids	3*n*-PCR
Mutungi et al., 2007 [4]	To study the effect of dietary cholesterol in the context of a low-carbohydrate intake on the mRNA expression of LDLR and HMGCR. To evaluate PBMCs as a surrogate for hepatic expression of these genes.	RCT	Carbohydrate-restricted diet (10–15% en) + either 640 mg/day additional dietary cholesterol from eggs (EGG group) or 0 mg/day of dietary cholesterol from egg substitutes (SUB group).	Twenty-eight overweight/obese individuals (15, EGG and 13, SUB)	Twelve weeks	Five-day food records	qRT-PCR, SYBR Green I chemistry
de Mello et al., 2008 [41]	To study if long-term weight loss changed mRNA expression levels of genes encoding inflammatory markers and if these changes were associated with changes in insulin and glucose metabolism.	RCT	Weight-reduction (WR) diet (AHA) with a mean deficit in caloric intake of 500 kJ/day (intervention) vs. individuals' usual diet (control).	Thirty-four overweight/obese individuals with abnormal glucose metabolism and features of the metabolic syndrome (24, intervention; ten, control)	Thirty-three weeks	Four-day food records	qRT-PCR, Taqman® chemistry
de Mello et al., 2008 [75]	To study if long-term weight loss changed mRNA expression levels of genes involved in the NFkB pathway and if these changes were associated with the improvement in insulin sensitivity (S_I).	Same as above	Same as above	Same as above	Thirty-three weeks	Same as above	Same as above
de Mello et al., 2009 [40]	To study the effect of a fatty fish (FF) or a lean fish (LF) diet on the modulation of inflammatory and endothelial function-related genes at mRNA level, and its association with serum FA profile and plasma metabolic compounds related to IR.	RCT	Diet recommended for coronary heart disease patients; control group < one meal of fish/week; FF group ≥ four meals of FF/week; LF group ≥ four meals of LF/week	Twenty-seven individuals with stable coronary heart disease (six, control; ten, FF; 11, LF)	Eight weeks	Serum FA composition in triglycerides, cholesterol esters, and phospholipids, and seven-day food records	qRT-PCR, Taqman® chemistry

Table 1. Continued

Study	Aim/purpose[a)]	Study design	Intervention(s)	Population[b)]	Intervention duration	Diet compliance	Methodology for the mRNA expression studies
Bouwens et al., 2009 [42]	To study the effects of long-term EPA + DHA supplementation on whole-genome gene expression profile.	Double-blind, RCT	Two-week placebo run-in period; intervention period: 0.4 g EPA + DHA/d (low EPA + DHA), 1.8 g EPA + DHA/d (high EPA + DHA), or 900 mg of high-oleic acid sunflower oil (control).	Fifty-one of the 111 elderly individuals (23, high EPA + DHA group; 25, control group)	Twenty-six weeks	Plasma FA composition in cholesterol esters	Microarray (Human whole-genome NuGO Genechip array; Affymetrix®)
				Hundred and eleven elderly individuals (37, low EPA + DHA group; 36, high EPA + DHA group; 38, control group)			qRT-PCR, Taqman® chemistry
Konstantinidou et al., 2010 [45]	To study whether a TMD and the polyphenols present in olive oil promote changes in the expression of atherosclerosis-related genes.	RCT	Traditional TMD + virgin olive oil (TMD + VOO group); TMD + washed VOO (TMD + WOO group); or the individuals' habitual diet (control group)	Fifty-six nonobese healthy individuals (20, TMD + VOO; 16, TMD + WOO; 20, control)	Three months	Urinary polyphenols present in the olive oils.	qRT-PCR, Taqman® chemistry
Bakker et al., 2010 [63]	To study the antiinflammatory effects induced by nutritional intervention ("antiinflammatory dietary mix") by using a nutrigenomics approach, including transcriptomics in PBMCs.	Double-blind, randomized, crossover, controlled study	"Antiinflammatory dietary mix" (AIDM group) capsules consisting of fish oil, green tea extract, resveratrol, vitamin E, vitamin C, and tomato extract vs. placebo capsules (placebo group)	Thirty-two healthy overweight/obese males with mild plasma hsCRP concentrations (1–10 mg/L).	Five weeks	Returned capsules, food diary, and plasma vitamin E	Microarray (NuGO Affymetrix Human Genechip NuGO_Hs1a520180; Affymetrix®)
Radler et al., 2011 [52]	To study the effects of low-dose of PUFA, polyphenols, and L-carnitine (PPC) as dietary supplements on the transcription of genes involved in FA metabolism.	Double-blind prospective, parallel, controlled study	Low-fat yogurt (125 g) containing low dose of grape seed extract, fish oil, phospholipids, L-carnitine, vitamins C, and E (PPC group), vs. a low-fat yogurt (125 g) containing low dose of vitamins C and E (control group)	Forty-two moderately hyperlipidemic obese individuals (22, PPC; 20, control)	Twelve weeks	Urinary carnitine and acylcarnitine	qRT-PCR, SYBR Green I chemistry
Wang et al., 2011 [61]	To study the effects of modification in the dietary fat: carbohydrate ratio and soy supplementation (SFD) on mRNA expression.	Participant-blinded, RCT	Very low fat diet (VLFD: 11% en fat, 68% en carbohydrate); a balanced diet (SFD: 30% en fat, 50% en carbohydrate) + SFD (50 mg isoflavonoids/day); or a control balanced diet (CD: with no SFD).	Fifty-eight healthy postmenopausal women not currently using menopausal hormone therapy (21, VLCD; 20, SFD; 17, CD).	Eight weeks	Daily food record and circulating levels of HDL cholesterol and triglycerides.	Microarray (Affymetrix Human Genome U95Av2 arrays; Affymetrix®)

Table 1. Continued

Study	Main results: mRNA expression profile in PBMCs	Main results: Other endpoints
Kaminski et al., 1993 [37]	PDGFA and PDGFB mRNA expression levels were lower at the end of the intervention than at baseline, and were higher at the end of the study than at baseline in the control group.	PDGFA and PDGFB mRNA expression levels were not detected in granulocytes.
Baumann et al., 1999 [38]	N-3 FAs intervention reduced CCL2, PDGFA, and PDGFB mRNA expression levels, while the other interventions and control group did not.	Similar results in ex vivo experiments.
Mutungi et al., 2007 [4]	HMGCR and LDLR mRNA expression levels decreased in the EGG group, and increased in the SUB group. Differences between the groups are not clearly stated.	No changes in total and LDL cholesterol.
de Mello et al., 2008	Decreased mRNA expression levels of TNF and IL1B in the WR group compared with the control group, the latter associated with the improvement in S_I. Within the WR group, additional decrease in IL1RN and IL18 and increase in IL8 and IL6 mRNA expression levels were observed.	Greater weight loss and decrease in fasting glucose levels in the WR group. Improvement in S_I and in serum antiinflammatory response within the WR group.
de Mello et al., 2008	A downregulation in genes involved in the transcription factor NFκB pathway within the WR group, where some of the changes were associated with the improvement in S_I.	Same as above.
de Mello et al., 2009	No changes in the mRNA expression levels of selected genes related to inflammation and endothelial function within or between the study groups.	Decrease in serum sICAM1 concentrations after the LF intervention. A decrease in serum AA:EPA ratio was associated with an antiinflammatory response at mRNA level in the FF group. A decrease in serum SFA proportion was positively associated with the change in sICAM1 mRNA expression in the LF group.
Bouwens et al., 2009	High EPA + DHA down-regulated genes involved in eicosanoid and prostaglandin syntheses, apoptosis, and adipogenesis differently from the control group. Within the high EPA + DHA group, about 15% of the PPARα target genes of interested were downregulated. qRT-PCR confirmed downregulation of the PPARα target genes in the high EPA + DHA group and a lesser downregulation in the low EPA + DHA group, both greater than in the control group.	Plasma hs-CRP did not change significantly in either of the intervention groups.
Konstantinidou et al., 2010 [45]	In the three-group analyses, TMD + VOO down-regulated genes related to the inflammation process (IFN-γ and ARHGAB15) and oxidative stress (ADRB2) compared with the control diet.	In the three-group analyses: Decrease in LDL cholesterol after TMD + VOO compared with both TMD + WOO and control diet. Decrease in hsCRP concentration after TMD + VOO compared with the control diet. In the within-group analyses: Decrease in total, HDL and LDL cholesterol after TMD + VOO. Decrease in $F_{2\alpha}$ isoprostanes, IFN-γ, and P-selectin concentrations after TMD + VOO intervention. Decrease in hsCRP concentrations after both TMD + VOO and TMD + WOO interventions.
Bakker et al., 2010 [63]	Genes related to inflammation were mostly downregulated. Favorable response from genes related to plaque formation and coagulation and blood cell differentiation.	Plasma concentrations of adiponectin were higher and of sICAM-1 and sVCAM-1 were lower at the end of the study in the AIDM than in the placebo group. Nutrigenomics approach involving transcriptomics, proteomics, metabolomics conclude an antiinflammatory and antioxidant effect of AIDM.
Radler et al., 2011 [52]	The mRNA expression levels of PPARα and selected target genes were downregulated at the end of the PPC but not in the control group.	Cell culture experiments in HepG2 cells produced similar results than in PBMCs. PPC intervention decreased plasma FFA and triglycerides levels.
Wang et al. 2011 [61]	Modest effects of the diets on gene expression response that were not different between the groups.	Modest (3%) weight loss and decrease in total cholesterol and leptin levels across the groups.

a) mRNA expression measured in PBMCs.
b) Included in PBMC studies.
3*n*-PCR, *n*on-overlapping *n*ested primers with *n*onlabeled detection PCR; AA, arachidonic acid; AHA, American heart Association; RCT, randomized, controlled trial; SFA, saturated fatty acid; TMD, traditional Mediterranean diet.

individuals were studied, which is a good sample size for confirming microarray findings.

Although not in a controlled trial setting, Weaver and collaborators [43] conducted a study in 27 healthy volunteers with the aim of studying the effects of changes in the dietary *n*-6/*n*-3 FAs ratio on genes related to early signal transduction of *n*-3 FA metabolites and genes encoding for cytokines and chemokines. The authors found that the 4-week supplementation period with borage and fish oils decreased the *n*-6/*n*-3 dietary ratio and reduced leukotriene production in leukocytes, which is a putative marker of inflammation. Along with these findings, this supplementation resulted in downregulation of IL23, IL10, and phosphatidylinositol 3-kinase γ (PI3Kγ) at the mRNA level in PBMCs. These findings indicated a role for an increased *n*-3 FA intake on modulating autoimmune and antiinflammatory responses. Moreover, these results provided some evidence that changes in gene expression are likely an important mechanism by which PUFA exert their potent effects in clinical conditions.

Consumption of virgin olive oil (VOO) rich in phenolic compounds and high in MUFA seem to have healthy benefits in humans, e.g. on heart disease risk factors [44]. A study exploring the mechanisms of the beneficial effect of VOO, a major component of a Mediterranean diet, on gene expression at mRNA levels in PBMCs was recently published [45] (Table 1). For this study, the authors used the candidate gene approach based on previous studies on gene expression response after acute VOO administration [46], and after a 3-wk single-arm virgin oil intervention study in ten healthy volunteers [47]. The latter showed that addition of 25 mL/day of VOO upregulated genes involved in the DNA repairing system, antiapoptotic genes, genes involved in antioxidant and oxidative cell defense mechanisms, PPAR-binding protein, and ADAM metallopeptidase domain 17 (ADAM17) using both microarray and qRT-PCR approaches. In all these studies, the authors aimed at studying the effect of VOO on gene expression from PBMCs to gain insights on the molecular mechanisms underlying the beneficial effect of VOO in the prevention of atherosclerosis. In the most recent study [45] (Table 1), the authors more specifically aimed to study the effects of VOO in a Mediterranean diet on atherosclerosis-related genes. Compared to the control diet, the traditional Mediterranean diet + VOO (TMD + VOO) down-regulated genes related to the inflammation process (IFN-γ and ARHGAP15: Rho GTPase activating protein 15) and oxidative stress (ADRB2: adrenergic β-2 receptor surface). Moreover, this intervention also reduced LDL cholesterol levels and decreased systemic inflammation (hsCRP concentration) compared with the control diet. The higher amount of urinary polyphenols (tyrosol and hydroxytyrosol), indicating compliance with VOO intake, was correlated with the expression of the IL17 receptor (IL17R) at the end of the study period. Also, the increase in the urinary tyrosol content was correlated with the decrease in IFN-γ expression specifically after the TMD + VOO intervention. The results derived from this study were relevant in the sense that a traditional Mediterranean diet might in fact exert its antiatherogenic properties in part through its effects on gene expression.

4 Dietary modifications and mRNA expression response associated with cholesterol and FA metabolism

4.1 Cholesterol metabolism

PBMCs and hepatic cells seem to share similarities in terms of cholesterol homeostasis. Therefore, immune cells may be a useful model system for liver lipid metabolism [48]. Some recent studies have assessed the effect of dietary cholesterol reintroduction after dietary fat restriction on mRNA expression of genes related to cholesterol homeostasis using PBMCs as a surrogate tissue for liver cells (hepatocytes). Results from two nonrandomized, short-term, controlled intervention studies have shown that dietary fat restriction and dietary cholesterol reintroduction resulted in changes in the mRNA expressions of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), LDL-receptor (LDLR), LDL receptor protein (LRP) and ethanol-inducible CYP2E1 [49, 50]. As novel findings, it was suggested that LRP, which changed in parallel with serum total and HDL cholesterol levels after reintroduction of dietary cholesterol, might also participate in the control of cholesterol synthesis. Moreover, CYP2E1 mRNA expression in PBMCs seemed to reflect the liver expression response to dietary cholesterol [49, 50].

In line with the results presented above, and also using PBMCs as a surrogate tissue for hepatic cells, dietary cholesterol in a low-carbohydrate diet context aiming at weight loss resulted in downregulation in these same genes regulating cholesterol homeostasis (HMGCR and LDLR) in young overweight healthy adults. In contrast, restrictions on dietary cholesterol increased their expression levels (Table 1) [4]. However, changes in total and LDL cholesterol levels were not observed.

Two other studies also reported results on the response at mRNA level of genes related to cholesterol metabolism using PBMCs as a surrogate tissue for hepatic cells [2, 51]. These studies related the beneficial effects of their experimental diets on the lipid profile (increase in the dietary carbohydrate–fat ratio for 3 weeks [2] or weight reduction through energy restriction, physical activity, and use of carnitine supplementation for 10 weeks [51]) with the modulation of genes from PBMCs related to cholesterol metabolism. Overall, in terms of diet, it seemed that the decreased intake of fat with a corresponding increase in carbohydrates for a 3-week period resulted in a better lipid, glucose, and insulin profile and in higher HMGCR expression than an isocaloric high-fat, low-carbohydrate diet [2]. Energy restriction over a 10-week period with a concomitant decrease in cholesterol intake resulted in a 5% weight loss and a decrease in total and LDL cholesterol,

triglycerides and plasma insulin, and upregulated LDLR expression [51].

4.2 FA metabolism

Also using PBMCs as a surrogate tissue for hepatic cells, a recent study explored the effects of a low-dose PUFA, polyphenols, and L-carnitine supplementation (PPC supplementation) on the mRNA expression of genes involved in FA metabolism [52] (Table 1). This intervention was based on the grounds that *n*-3 FAs can induce gene expression via PPARs [53], and that along with L-carnitine, both may modulate mRNA expression of genes involved in lipid metabolism in experimental models [54, 55]. Complementary studies in cell culture were also performed (Table 1). Twelve weeks of PPC supplementation decreased plasma levels of free FAs and triglycerides and upregulated PPARα (PPARA) and selected target genes involved in FA oxidation (CPT1A: carnitine palmitoyltransferase 1A and CPT1B: carnitine palmitoyltransferase 1B). Similar results were also found in the hepatic cell line (HepG2 cells). These findings support the use of PBMCs as surrogate for hepatic cells for the study of CPT1A and CPT1B.

5 Dietary modifications based on dietary antioxidant and antiinflammatory properties and mRNA expression response

Another great interest is the elucidation of mechanisms dealing with the beneficial effects of foods with antioxidant capacity, e.g. fruits and vegetables, on health and how much these benefits are related to reduced oxidative stress. Consumption of these foods may reduce the presence of factors related to the metabolic syndrome [56–58], and have also been associated with an antiinflammatory profile and improved antioxidant capacity [59, 60].

Not many dietary intervention studies have been conducted aiming at studying the effects of antioxidant dietary components on PBMC mRNA expression profile. However, some studies referred in this review have used some of these food components in the interventions, e.g. grape extracts (162 mg per day) in combination with other sources of polyphenols with antioxidant properties, *n*-3 PUFA and L-carnitine [52] or virgin oil in the traditional Mediterranean diet [45].

In a recent work conducted by Wang et al., the authors aimed at evaluating the potential of gene expression profiling in PBMCs after dietary fat reduction or soy supplementation. The aim was to understand the molecular basis for the potential benefits of reduced fat intake and soy supplementation on health, e.g. on prevention and treatment of breast cancer. In this study, only modest and similar changes in the gene expression response after the experimental and control diets (Table 1) were observed. However, within-group analyses revealed that the gene encoding nicotinamide phosphoribosyltransferase or "visfatin" (NAMPT) was significantly downregulated after reducing dietary fat and increasing carbohydrate in the diet or after following a balanced diet regimen supplemented with soy food [61]. In addition, the gene pathway analyses showed that these experimental diets reduced the mRNA expression of genes involved in Fc γ mediated phagocyosis and in cytokine receptor interactions. Most of these genes were strongly associated with NAMPT, and none of the findings were mediated by weight loss. This was an interesting finding because visfatin seems to be inversely associated with adiposity and IR in obesity and T2DM [62].

With a different perspective than the studies described above and trying to apply a global "omics" approach, Bakker et al. conducted an intervention trial for studying the effects of an "antiinflammatory diet mix" (AIDM) consisting of a combination of polyphenols, antioxidants, and antiinflammatory components on outcome measurements from the blood circulation, PBMCs, and adipose tissue cells [63] (Table 1). The global gene expression profile of PBMCs was used as a surrogate for hepatic cells.

This study was conducted in young and middle-aged relatively healthy persons. The authors observed that the antiinflammatory and antioxidant effect of the AIDM was reflected in PBMCs by modulation of certain genes related to immune-inflammatory response and blood cell differentiation (IGHD: immunoglobulin heavy constant delta, MYOM1: myomesin 1, IGHV3-47: immunoglobulin heavy variable 3-47, and the IL12A, IL4R: IL4 receptor, CCL21: and chemokine C-C motif ligand 21), and to oxidative stress (LACTB: lactamase, β). The suggested beneficial effect of the AIDM on plaque formation (adipose tissue transcriptomic analyses) and lipid metabolism (lipidomic and metabolomic analyses) was also reflected in PBMCs at the level of mRNA expression. It is important to keep in mind that this was an intervention study in which dietary manipulation was done by means of supplementation with no details on the background and follow-up of dietary intake. The authors stated that this study might be considered, though, as a proof of concept.

This study highlights and strengthens the use of the "omics" approach, which combines metabolomics, proteinomics, and gene profiling to better describe the molecular mechanisms of dietary changes. It also reinforces that the "omics" approach can help to elucidate the influence of dietary changes on inflammation, oxidative stress, and metabolism. PBMCs in this study more likely added complementary information to the findings from the adipose tissue transcriptomics [63] rather than reflected changes in the same genes in these two target tissues. Therefore, information coming from both adipose tissue and PBMC transcriptomics contributed to the biological interpretation of the beneficial effect of the dietary supplementation, as e.g. on endothelial function and vascular health.

6 Diet-induced weight loss is associated with immune-inflammatory and antioxidant responses and modulation of mRNA expression

Overweight and obesity substantially increase the risk of morbidity from hypertension, dyslipidemia, coronary artery disease, and T2DM. A high body mass index is also associated with an increase in all-cause mortality [64, 65]. Management of obesity consists of weight loss and monitoring of metabolic risk factors. Weight loss is also a strategy for treating these metabolic risk factors [64, 65]. Persons at high risk for developing T2DM should also benefit from weight reduction, as shown, e.g. in the Finnish Diabetes Prevention Study and Diabetes Prevention Program [66, 67]. Weight reduction has also been shown to reduce both low-grade inflammation [68, 69] and to some extent markers of endothelial dysfunction [70].

Results coming from three single-arm intervention studies aiming at reducing body weight through energy restriction were published in the year 2008. In these studies, PBMCs were utilized as an alternative to adipose tissue cells to study oxidative stress and inflammatory status [71–73]. One of the studies was conducted in nine obese otherwise healthy middle-aged men who followed a balanced 8-wk low-caloric diet resulting in a weight loss of about 9% [71]. The authors observed a concomitant decrease in the mRNA expression of specific genes involved in the process of oxidative stress and inflammation. This study was a pioneer on assessing the usefulness of the microarray approach in PBMCs to detect molecular changes after a diet-induced weight loss in overweight or obese subjects. In a similar dietary intervention study, caloric restriction also upregulated the expression of two specific genes encoding sirtuins (SIRT1 and SIRT2), which in combination represented the "global" sirtuin mRNA expression [72]. Along with the changes in the gene expression levels, caloric restriction also resulted in 6% reduction on body weight, decreased blood total cholesterol levels, and improved antioxidant capacity (biomarkers measured in the circulation). The changes in the mRNA expression levels of SIRT1 and SIRT2 were not, however, associated with the changes in body weight. In this particular study, PBMCs were also used as an alternative to skeletal muscle tissue.

In another study with similar design, 21 volunteers who underwent a low-caloric diet for a 12-wk period lost 5% of their initial body weight and also had a decrease of about 30% in the mRNA expression of proinflammatory markers (TNF: tumor necrosis factor, IL6, MIF: macrophage migration inhibitory factor, and MMP9: matrix metallopeptidase 9) in PBMCs. A decrease in the NFκB activity in these cells was also observed [73]. Moreover, an improvement in fasting insulin and glucose levels, a better plasma lipid profile, and reduced circulating levels of proinflammatory markers, including hsCRP occurred.

Following these studies, in a randomized controlled design, our research group has shown that in the circulating PBMCs of overweight individuals with the metabolic syndrome and abnormal glucose metabolism, modulation of genes encoding pro- and antiinflammatory markers occurred after weight loss, coupled with an improvement in glucose and insulin metabolism [41] (Table 1). In this study, the aim of the dietary advice was healthy diet with a minimum energy deficit of 500 kJ/day (Table 1). PBMC gene expression was utilized as a model for studying the inflammatory response and its relationship with IR as possible underlying mechanisms of diseases such as T2DM. In this study, the mRNA expression levels of the proinflammatory marker genes IL1β (IL1B) and TNF responded differently between the weight reduction and control groups (Table 1). Although we did not find a significant difference between the experimental and control groups in the expression of other studied genes (Table 1), the antiinflammatory response achieved after 9 months of dietary advice aiming at losing weight (5%) resulted in up- and downregulation of other genes encoding cytokines (Table 1). An interesting finding was that while no associations between the changes in body composition were found with the changes in gene expression, the increase in S_I measured with a frequent sampled intravenous glucose tolerance test was associated with the decrease in the IL1B mRNA expression in participants following the diet-weight loss intervention. Post-hoc analyses also showed that the mRNA expression of TNF, IL6, and IL1B in PBMCs was positively correlated with the mRNA expression of ghrelin, during the study [74]. It is possible that factors other than the weight reduction itself, such as dietary changes, played a role in the response of the genes studied.

Further studies in the same study population have shown that in PBMCs, the expression of genes encoding receptors of surface ligands involved in the NFκB pathway, which is implicated in the regulation of inflammatory responses and control of the innate immune system, was attenuated and associated with the improvement of S_I in the diet-weight loss intervention [75]. Some of the receptors associated with the improvement of S_I, e.g. toll-like receptor (TLR) 4, are part of the bacterial LPS receptor that may play a role in IR [76]. These receptors can sense dietary components such as PUFA and saturated fat and control activation of inflammatory pathways [76]. Again we could not observe any direct association between the changes in body composition and in the mRNA expression of these receptors, and there was no attenuation of the results when they were adjusted for the intake of major dietary nutrients. Nevertheless, we found that the improvement of S_I was associated with the downregulation of TLR2 and TLR4, CCL5 and tumor necrosis factor receptor superfamily, member 1A (TNFRSF1). Therefore, based on all these findings, we suggested that the study of the mRNA expression in PBMCs can provide information about genes involved in IR states and perhaps also lead to finding responsive molecules or signatures that could help on the

process of preventing diet-related diseases such as T2DM and CVDs.

7 Concluding remarks

Various types of dietary FA supplementations, mostly *n*-3 FAs, and their effects on the mRNA gene expression response in PBMCs have been studied. These data provide evidence that changes in gene expression are likely an important mechanism by which PUFA exert their potent effects on clinical conditions such as atherosclerosis and CVDs. Based on the studies presented in this review, it seems that the best evidence on long-chain FA supplementation is that fish oil, in elderly individuals, alter gene expression at mRNA levels in PBMCs to a more antiinflammatory and antiatherogenic direction. The effect of fish intake, however, deserves further investigation. An interesting finding was also that the use of VOO in a Mediterranean diet might exert its antiatherogenic properties in part through its effects on gene expression. However, either the lack of (i) appropriate sample size [37], (ii) using statistical methods to evaluate the outcomes [37], (iii) of making additional statistical procedures to refine the comparisons between the study groups [38,45], or (iv) control group and proper wash-out period [43] limited a better understanding of the results presented from the studies involving dietary changes of long-chain *n*-3 FAs. Moreover, the use of different procedures for isolating PBMCs among the studies (traditional Ficoll density gradient separation (Ficoll) vs. the newest cell preparation tubes (CPT)) could also have lead to sample contamination, as e.g. the presence of granulocytes in the PBMC sample [77]. It is not known, however, how exactly this could have interfered in the observed results at the gene expression levels.

On the other hand, based on the studies using dietary interventions testing PBMCs as a surrogate tissue for hepatic cell mRNA gene expression, it seemed that these circulating immune cells do reflect cholesterol metabolism regulation at the molecular level with respect to HMGCR and LDLR. However, lack of control group and modern methodology for assessing gene expression in most of the studies also warrant new studies to reproduce these findings. Different methods for isolating the PBMC among the studies are also a shortcome. Other types of dietary interventions [52] not only emphasizing changes in dietary cholesterol and fat/carbohydrate ratio intakes should also be used in transcriptomic studies.

Synergistic effects of dietary components in a high-dose supplementation on ameliorating oxidative stress and inflammation resulting in modulation of genes related to immune-inflammatory response and blood cell differentiation have been described. However, in studies where the beneficial effect of more feasible doses of supplementation of antioxidant foods were given (e.g. soy), no clear effect of the dietary intervention on PBMC transcriptomics was found.

Modulation of genes related to inflammatory responses is the most common result encountered in gene expression studies in PBMCs after diet-induced weight loss. Direct associations found between these responses at transcriptional level and clinical variables related to glucose and insulin metabolism after weight loss also indicated some synchronized response between PBMC gene expression and risk factors. The weight loss achieved in these dietary interventions, however, also improved the same clinical outcomes and others, such as the lipid profile. In this sense, the effect of weight loss per se should be always taken into account in nutrigenomic studies, because it can be a confounding factor when estimating the effect of the diet. Other limitations such as lack of control group or data on genome-wide transcriptomics, use of different procedures among the studies for isolating the PBMCs and very small sample size have limited broader perspectives, especially when it comes for the use of PBMCs as a surrogate tissue for adipose tissue or skeletal muscle cells. Nevertheless, the finding that PBMCs are modulated by energy restriction and weight loss is interesting and promising. Genes modulated by these changes and the associated pathways were related to processes involved in inflammatory responses and IR, which are linked to conditions such as the metabolic syndrome, T2DM, and CVD.

Currently, PBMCs seem to reflect the liver environment and complement adipose tissue findings in transcriptomics. PBMC gene expression after dietary intervention studies can be used for studying the response of genes related to PPARα pathway and cholesterol metabolism such as HMGCR and LDLR. They can also be used to explore the effect of dietary interventions on inflammation, even though not all the interventions that modulated inflammation-related genes resulted in changes of markers of low-grade inflammation. Moreover, PBMC transcriptomics seem to reflect dietary interventions or weight loss resulting in a better insulin and glucose metabolism possibly also at the skeletal muscle cell level. However, PBMC transcriptomics from dietary intervention studies have not yet resulted in clear confirmation of candidate genes related to disease risk.

In summary, PBMCs have been used only in a few randomized, controlled intervention studies in humans to assess the effect of dietary manipulations. Therefore, little is known about the interface between the molecular profile in the PBMCs and metabolic responses to dietary interventions or the concordance of gene modulation with that in other tissues. Although high-throughput methodologies are costly, professional centers with experienced personnel have been established to facilitate the use of the whole transcritomics approach to better characterize the effect of various dietary interventions. The careful planning of study designs, use of standardized, reliable, and valid methods for sample handling, PBMC isolation and RNA extraction, and clearer reporting of the methodology used are critical issues when planning and conducting transcriptomic studies to avoid lack of study power, inaccurate results, and loss of valuable material. Even after overcoming these hurdles, it would be still possible that gene expression response could also vary according to the proportion of cell type that make up PBMCs. Overall, PBMCs

seem to be a suitable tissue to be explored in nutrigenetic studies and a promising tool in the field of nutrigenomics.

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8 References

[1] Parihar, P., *Microbiology and Immunology*, Global Media, Delhi 2009.

[2] Vidon, C., Boucher, P., Cachefo, A., Peroni, O. et al., Effects of isoenergetic high-carbohydrate compared with high-fat diets on human cholesterol synthesis and expression of key regulatory genes of cholesterol metabolism. *Am. J. Clin. Nutr.* 2001, *73*, 878–884.

[3] Auboeuf, D., Vidal, H., The use of the reverse transcription-competitive polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. *Anal. Biochem.* 1997, *245*, 141–148.

[4] Mutungi, G., Torres-Gonzalez, M., McGrane, M. M., Volek, J. S., Fernandez, M. L., Carbohydrate restriction and dietary cholesterol modulate the expression of HMG-CoA reductase and the LDL receptor in mononuclear cells from adult men. *Lipids Health Dis.* 2007, *6*, 34.

[5] Moody, D. E., Genomics techniques: an overview of methods for the study of gene expression. *J. Anim. Sci.* 2001, *79*, E128–E135.

[6] Wittwer, J., Rubio-Aliaga, I., Hoeft, B., Bendik, I. et al., Nutrigenomics in human intervention studies: current status, lessons learned and future perspectives. *Mol. Nutr. Food Res.* 2011, *55*, 341–358.

[7] Kallio, P., Kolehmainen, M., Laaksonen, D. E., Kekalainen, J. et al., Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT study. *Am. J. Clin. Nutr.* 2007, *85*, 1417–1427.

[8] Muller, M., Kersten, S., Nutrigenomics: goals and strategies. *Nat. Rev. Genet.* 2003, *4*, 315–322.

[9] American Diabetes Association, Executive summary: standards of medical care in diabetes–2011. *Diabetes Care* 2011, *34* (Suppl 1), S4–S10.

[10] Graham, I., Atar, D., Borch-Johnsen, K., Boysen, G. et al., European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur. Heart J.* 2007, *28*, 2375–2414.

[11] Powell, E. E., Kroon, P. A., Low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in human mononuclear leukocytes is regulated coordinately and parallels gene expression in human liver. *J. Clin. Invest.* 1994, *93*, 2168–2174.

[12] Ziegler-Heitbrock, H. W., Definition of human blood monocytes. *J. Leukoc. Biol.* 2000, *67*, 603–606.

[13] Fuchs, D., Piller, R., Linseisen, J., Daniel, H. et al., The human peripheral blood mononuclear cell proteome responds to a dietary flaxseed-intervention and proteins identified suggest a protective effect in atherosclerosis. *Proteomics* 2007, *7*, 3278–3288.

[14] Di Paolo, S., Schena, A., Stallone, G., Grandaliano, G. et al., Captopril enhances transforming growth factor (TGF)-beta1 expression in peripheral blood mononuclear cells: a mechanism independent from angiotensin converting enzyme inhibition? A study in cyclosporine-treated kidney-transplanted patients. *Transplantation* 2002, *74*, 1710–1715.

[15] Libby, P., Inflammation and cardiovascular disease mechanisms. *Am. J. Clin. Nutr.* 2006, *83*, 456S–460S.

[16] Kim, J. A., Montagnani, M., Koh, K. K., Quon, M. J., Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006, *113*, 1888–1904.

[17] Donath, M. Y., Shoelson, S. E., Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 2011, *11*, 98–107.

[18] Ghanim, H., Aljada, A., Daoud, N., Deopurkar, R. et al., Role of inflammatory mediators in the suppression of insulin receptor phosphorylation in circulating mononuclear cells of obese subjects. *Diabetologia* 2007, *50*, 278–285.

[19] Fogeda, M., Gallart, L., Gutierrez, C., Vendrell, J. et al., High expression of tumor necrosis factor alpha receptors in peripheral blood mononuclear cells of obese type 2 diabetic women. *Eur. Cytokine Netw.* 2004, *15*, 60–66.

[20] Hansson, G. K., Inflammation, atherosclerosis, and coronary artery disease. *N. Engl. J. Med.* 2005, *352*, 1685–1695.

[21] Bomprezzi, R., Ringner, M., Kim, S., Bittner, M. L. et al., Gene expression profile in multiple sclerosis patients and healthy controls: identifying pathways relevant to disease. *Hum. Mol. Genet.* 2003, *12*, 2191–2199.

[22] Rokutan, K., Morita, K., Masuda, K., Tominaga, K. et al., Gene expression profiling in peripheral blood leukocytes as a new approach for assessment of human stress response. *J. Med. Invest.* 2005, *52*, 137–144.

[23] van de Vijver, M. J., He, Y. D., van't Veer, L. J., Dai, H. et al., A gene-expression signature as a predictor of survival in breast cancer. *N. Engl. J. Med.* 2002, *347*, 1999–2009.

[24] Connolly, P. H., Caiozzo, V. J., Zaldivar, F., Nemet, D. et al., Effects of exercise on gene expression in human peripheral blood mononuclear cells. *J. Appl. Physiol.* 2004, *97*, 1461–1469.

[25] Dabek, J., Kulach, A., Wilczok, T., Mazurek, U. et al., Transcriptional activity of genes encoding interferon gamma (IFNgamma) and its receptor assessed in peripheral blood mononuclear cells in patients with cardiac syndrome X. *Inflammation* 2007, *30*, 125–129.

[26] Ma, J., Dempsey, A. A., Stamatiou, D., Marshall, K. W., Liew, C. C., Identifying leukocyte gene expression patterns associated with plasma lipid levels in human subjects. *Atherosclerosis* 2007, *191*, 63–72.

[27] Wettinger, S. B., Doggen, C. J., Spek, C. A., Rosendaal, F. R., Reitsma, P. H., High throughput mRNA profiling highlights associations between myocardial infarction and aberrant expression of inflammatory molecules in blood cells. *Blood* 2005, *105*, 2000–2006.

[28] Dandona, P., Aljada, A., Mohanty, P., Ghanim, H. et al., Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J. Clin. Endocrinol. Metab.* 2001, *86*, 3257–3265.

[29] Hotamisligil, G. S., Inflammation and metabolic disorders. *Nature* 2006, *444*, 860–867.

[30] Rudkowska, I., Raymond, C., Ponton, A., Jacques, H. et al., Validation of the use of peripheral blood mononuclear cells as surrogate model for skeletal muscle tissue in nutrigenomic studies. *OMICS* 2011, *15*, 1–7.

[31] Emilsson, V., Thorleifsson, G., Zhang, B., Leonardson, A. S. et al., Genetics of gene expression and its effect on disease. *Nature* 2008, *452*, 423–428.

[32] Goring, H. H., Curran, J. E., Johnson, M. P., Dyer, T. D. et al., Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. *Nat. Genet.* 2007, *39*, 1208–1216.

[33] Freedman, J. E., Larson, M. G., Tanriverdi, K., O'Donnell, C. J. et al., Relation of platelet and leukocyte inflammatory transcripts to body mass index in the Framingham heart study. *Circulation* 2010, *122*, 119–129.

[34] Kolehmainen, M., Salopuro, T., Schwab, U. S., Kekalainen, J. et al., Weight reduction modulates expression of genes involved in extracellular matrix and cell death: the GENOBIN study. *Int. J. Obes. (Lond.)* 2008, *32*, 292–303.

[35] Tolppanen, A. M., Pulkkinen, L., Kolehmainen, M., Schwab, U. et al., Tenomodulin is associated with obesity and diabetes risk: the Finnish diabetes prevention study. *Obesity* 2007, *15*, 1082–1088.

[36] Tolppanen, A. M., Pulkkinen, L., Herder, C., Koenig, W. et al., The genetic variation of the tenomodulin gene (TNMD) is associated with serum levels of systemic immune mediators—the Finnish Diabetes Prevention Study. *Genet. Med.* 2008, *10*, 536–544.

[37] Kaminski, W. E., Jendraschak, E., Kiefl, R., von Schacky, C., Dietary omega-3 fatty acids lower levels of platelet-derived growth factor mRNA in human mononuclear cells. *Blood* 1993, *81*, 1871–1879.

[38] Baumann, K. H., Hessel, F., Larass, I., Muller, T. et al., Dietary omega-3, omega-6, and omega-9 unsaturated fatty acids and growth factor and cytokine gene expression in unstimulated and stimulated monocytes. A randomized volunteer study. *Arterioscler. Thromb. Vasc. Biol.* 1999, *19*, 59–66.

[39] Niu, J., Kolattukudy, P. E., Role of MCP-1 in cardiovascular disease: molecular mechanisms and clinical implications. *Clin. Sci. (Lond.)* 2009, *117*, 95–109.

[40] de Mello, V. D., Erkkila, A. T., Schwab, U. S., Pulkkinen, L. et al., The effect of fatty or lean fish intake on inflammatory gene expression in peripheral blood mononuclear cells of patients with coronary heart disease. *Eur. J. Nutr.* 2009, *48*, 447–455.

[41] de Mello, V. D., Kolehmainen, M., Schwab, U., Mager, U. et al., Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome. *Metabolism* 2008, *57*, 192–199.

[42] Bouwens, M., van de Rest, O., Dellschaft, N., Bromhaar, M. G. et al., Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. *Am. J. Clin. Nutr.* 2009, *90*, 415–424.

[43] Weaver, K. L., Ivester, P., Seeds, M., Case, L. D. et al., Effect of dietary fatty acids on inflammatory gene expression in healthy humans. *J. Biol. Chem.* 2009, *284*, 15400–15407.

[44] Covas, M. I., Nyyssonen, K., Poulsen, H. E., Kaikkonen, J. et al., The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann. Intern. Med.* 2006, *145*, 333–341.

[45] Konstantinidou, V., Covas, M. I., Munoz-Aguayo, D., Khymenets, O. et al., In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. *FASEB J.* 2010, *24*, 2546–2557.

[46] Konstantinidou, V., Khymenets, O., Fito, M., De La Torre, R. et al., Characterization of human gene expression changes after olive oil ingestion: an exploratory approach. *Folia Biol. (Praha)* 2009, *55*, 85–91.

[47] Khymenets, O., Fito, M., Covas, M. I., Farre, M. et al., Mononuclear cell transcriptome response after sustained virgin olive oil consumption in humans: an exploratory nutrigenomics study. *OMICS* 2009, *13*, 7–19.

[48] Bouwens, M., Afman, L. A., Muller, M., Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells. *Am. J. Clin. Nutr.* 2007, *86*, 1515–1523.

[49] Boucher, P., de Lorgeril, M., Salen, P., Crozier, P. et al., Effect of dietary cholesterol on low density lipoprotein-receptor, 3-hydroxy-3-methylglutaryl-CoA reductase, and low density lipoprotein receptor-related protein mRNA expression in healthy humans. *Lipids* 1998, *33*, 1177–1186.

[50] Boucher, P., Seree, E., Vidon, C., de Souza, A. C. et al., Dietary lipids affect human ethanol-inducible CYP2E1 gene expression in vivo in mononuclear cells. *Life Sci.* 2000, *67*, 1307–1316.

[51] Patalay, M., Lofgren, I. E., Freake, H. C., Koo, S. I. et al., The lowering of plasma lipids following a weight reduction program is related to increased expression of the LDL receptor and lipoprotein lipase. *J. Nutr.* 2005, *135*, 735–739.

[52] Radler, U., Stangl, H., Lechner, S., Lienbacher, G. et al., A combination of (omega-3) polyunsaturated fatty acids, polyphenols and L-carnitine reduces the plasma lipid levels and increases the expression of genes involved in fatty

acid oxidation in human peripheral blood mononuclear cells and HepG2 cells. *Ann. Nutr. Metab.* 2011, *58*, 133–140.

[53] Bunger, M., Hooiveld, G. J., Kersten, S., Muller, M., Exploration of PPAR functions by microarray technology—a paradigm for nutrigenomics. *Biochim. Biophys. Acta.* 2007, *1771*, 1046–1064.

[54] van Schothorst, E. M., Flachs, P., Franssen-van Hal, N. L., Kuda, O. et al., Induction of lipid oxidation by polyunsaturated fatty acids of marine origin in small intestine of mice fed a high-fat diet. *BMC Genomics* 2009, *10*, 110.

[55] Karlic, H., Lohninger, S., Koeck, T., Lohninger, A., Dietary l-carnitine stimulates carnitine acyltransferases in the liver of aged rats. *J. Histochem. Cytochem.* 2002, *50*, 205–212.

[56] Heidemann, C., Scheidt-Nave, C., Richter, A., Mensink, G. B., Dietary patterns are associated with cardiometabolic risk factors in a representative study population of German adults. *Br. J. Nutr.* 2011, *106*, 1253–1262.

[57] Kouki, R., Schwab, U., Hassinen, M., Komulainen, P. et al., Food consumption, nutrient intake and the risk of having metabolic syndrome: the DR's EXTRA Study. *Eur. J. Clin. Nutr.* 2011, *65*, 368–377.

[58] Esmaillzadeh, A., Kimiagar, M., Mehrabi, Y., Azadbakht, L. et al., Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. *Am. J. Clin. Nutr.* 2006, *84*, 1489–1497.

[59] Abete, I., Goyenechea, E., Zulet, M. A., Martinez, J. A., Obesity and metabolic syndrome: potential benefit from specific nutritional components. *Nutr. Metab. Cardiovasc. Dis.* 2011, *21* (Suppl 2), B1–B15.

[60] Hermsdorff, H. H., Zulet, M. A., Puchau, B., Martinez, J. A., Fruit and vegetable consumption and proinflammatory gene expression from peripheral blood mononuclear cells in young adults: a translational study. *Nutr. Metab. (Lond.)* 2010, *7*, 42.

[61] Wang, J., Siegmund, K., Tseng, C. C., Lee, A. S. et al., Soy food supplementation, dietary fat reduction and peripheral blood gene expression in postmenopausal women−−a randomized, controlled trial. *Mol. Nutr. Food. Res.* 2011, *55* (Suppl 2), S264–S277.

[62] Maury, E., Brichard, S. M., Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol. Cell. Endocrinol.* 2010, *314*, 1–16.

[63] Bakker, G. C., van Erk, M. J., Pellis, L., Wopereis, S. et al., An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *Am. J. Clin. Nutr.* 2010, *91*, 1044–1059.

[64] National Institutes of Health, National Heart, Lung and Blood Institute. The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults 2000, (NIH Publication No. 00-4084).

[65] World Health Organisation, *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. WHO Technical Report Series, 894*, World Health Organisation, Geneva, Switzerland 2000.

[66] Tuomilehto, J., Lindström, J., Eriksson, J. G., Valle, T. T. et al., Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* 2001, *344*, 1343–1350.

[67] Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F. et al., Diabetes Prevention Program Research Group, reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* 2002, *346*, 393–403.

[68] Selvin, E., Paynter, N. P., Erlinger, T. P., The effect of weight loss on C-reactive protein: a systematic review. *Arch. Intern. Med.* 2007, *167*, 31–39.

[69] Forsythe, L. K., Wallace, J. M., Livingstone, M. B., Obesity and inflammation: the effects of weight loss. *Nutr. Res. Rev.* 2008, *21*, 117–133.

[70] Kerr, S. M., Livingstone, M. B., McCrorie, T. A., Wallace, J. M., Endothelial dysfunction associated with obesity and the effect of weight loss interventions. *Proc. Nutr. Soc.* 2011, *70*, 418–425.

[71] Crujeiras, A. B., Parra, D., Milagro, F. I., Goyenechea, E. et al., Differential expression of oxidative stress and inflammation related genes in peripheral blood mononuclear cells in response to a low-calorie diet: a nutrigenomics study. *OMICS* 2008, *12*, 251–261.

[72] Crujeiras, A. B., Parra, D., Goyenechea, E., Martinez, J. A., Sirtuin gene expression in human mononuclear cells is modulated by caloric restriction. *Eur. J. Clin. Invest.* 2008, *38*, 672–678.

[73] Sheu, W. H., Chang, T. M., Lee, W. J., Ou, H. C. et al., Effect of weight loss on proinflammatory state of mononuclear cells in obese women. *Obesity (Silver Spring)* 2008, *16*, 1033–1038.

[74] Mager, U., Kolehmainen, M., de Mello, V. D., Schwab, U. et al., Expression of ghrelin gene in peripheral blood mononuclear cells and plasma ghrelin concentrations in patients with metabolic syndrome. *Eur. J. Endocrinol.* 2008, *158*, 499–510.

[75] de Mello, V. D., Kolehmainen, M., Pulkkinen, L., Schwab, U. et al., Downregulation of genes involved in NF kappaB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: the GENOBIN study. *Diabetologia* 2008, *51*, 2060–2067.

[76] Schenk, S., Saberi, M., Olefsky, J. M., Insulin sensitivity: modulation by nutrients and inflammation. *J. Clin. Invest.* 2008, *118*, 2992–3002.

[77] Schlenke, P., Kluter, H., Muller-Steinhardt, M., Hammers, H. J. et al., Evaluation of a novel mononuclear cell isolation procedure for serological HLA typing. *Clin. Diagn. Lab. Immunol.* 1998, *5*, 808–813.

