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Activation of peroxisome proliferator-activated receptor alpha in human peripheral blood mononuclear cells reveals an individual gene expression profile response

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Abstract

Background: Peripheral blood mononuclear cells (PBMCs) are relatively easily obtainable cells in humans. Gene expression profiles of PBMCs have been shown to reflect the pathological and physiological state of a person. Recently, we showed that the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) has a functional role in human PBMCs during fasting. However, the extent of the role of PPARα in human PBMCs remains unclear. In this study, we therefore performed gene expression profiling of PBMCs incubated with the specific PPARα ligand WY14,643.

Results: Incubation of PBMCs with WY14,643 for 12 hours resulted in a differential expression of 1,373 of the 13,080 genes expressed in the PBMCs. Gene expression profiles showed a clear individual response to PPARα activation between six healthy human blood donors. Pathway analysis showed that genes in fatty acid metabolism, primarily in β-oxidation were up-regulated upon activation of PPARα with WY14,643, and genes in several amino acid metabolism pathways were down-regulated.

Conclusion: This study shows that PPARα in human PBMCs regulates fatty acid and amino acid metabolism. In addition, PBMC gene expression profiles show individual responses to WY14,643 activation. We showed that PBMCs are a suitable model to study changes in PPARα activation in healthy humans.

Background

The function of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) has been studied extensively from the time of its discovery in the early 1990s [1]. PPARα is a ligand activated nuclear receptor, which is known to be activated by free fatty acids and their derivatives [2,3]. Besides fatty acids, several synthetic compounds are available that specifically activate PPARα, including hypolipidemic drugs, such as fibrates and pirinixic acid (WY14,643) [4]. Synthetic PPARα agonists mimic effects of dietary unsaturated fatty acids on hepatic gene expression in terms of regulation of target genes and molecular mechanism [5]. Activation of PPARα with WY14,643 in mice showed that the main function of PPARα in liver is the regulation of lipid metabolism, and more specifically fatty acid β-oxidation [6]. PPARα was

also found to be involved in regulation of amino acid metabolism [7] and inflammation [8,9]. In humans, the function of PPARα is examined less thoroughly, because functional studies are more complicated. There is no human genetic disease linked to a dysfunctional *PPARα* gene and tissue sampling is often not feasible in healthy volunteers. Blood is one of the few tissues which is readily available in healthy humans. Peripheral blood mononuclear cells (PBMCs) are relatively easily obtainable by isolation from blood. These cells consist of lymphocytes and monocytes/macrophages and it is known that PPARα is expressed in these cells [10,11]. The use of PBMCs has proven to be highly robust in distinguishing a disease state from healthy state, by studying gene expression profiles of these cells [12,13]. Recently, we showed that PBMC gene expression profiles of healthy volunteers can also reflect changes between 24 and 48 hours fasting, when plasma fatty acid concentrations are elevated. In addition, we showed that PPARα seems to have a functional role in human PBMC during fasting as several of the genes changed upon fasting were also changed upon incubation of PBMC with the specific PPARα agonist WY14,643 [14]. However, the extent of the role of PPARα in human PBMCs remains unclear. Therefore, we tried to elucidate the function of PPARα in human PBMCs by whole genome microarray analysis of the PBMCs incubated with the specific PPARα ligand WY14,643. Furthermore, to examine the complete role of PPARα within PBMCs during fasting, we compared microarray analysis of PBMCs activated with WY14,643, with microarray analysis of PBMCs during 24 hours of fasting.

Results

PPARα regulation in PBMCs after incubation with WY14,643

Incubation of PBMC with the specific PPARα ligand WY14,643 for 12 hours resulted in a differential expression of 1,373 of the 13,080 genes expressed in the PBMCs, indicating a PPARα-dependent regulation of 10.5% of the genes expressed in PBMC (Figure 1). More than half of these genes (56%) were up-regulated. Pathway analysis of the genes changed upon activation of PPARα with WY14,643, showed a marked increase in fatty acid metabolism, primarily in β-oxidation, and a decrease in several amino acid metabolism pathways (data not shown).

A peroxisome proliferator response elements (PPREs) was ascribed to 106 out of the 1,373 genes changed, using the study of Lemay *et al* [15]. Of these genes, 75 were up-regulated and 31 were down-regulated (Figure 2). Figure 2 shows the responses to activation of PPARα for each person by illustrating the changes in gene expression of these 106 genes per individual. For several genes a clear variation in response upon PPARα activation between individuals is present. Donor B and, especially, donor E show an

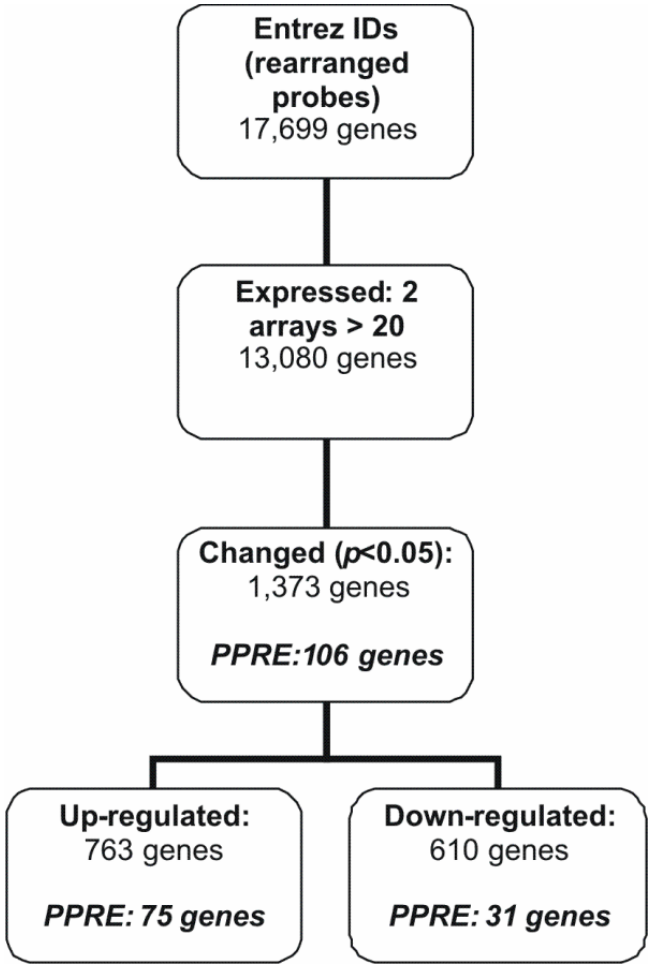

Figure 1
Gene selection procedure after microarray analysis of WY14,643 incubated PBMCs. Flow chart of the followed gene selection procedure after microarray analysis of WY14,643 incubated PBMCs from 6 donors. PPRE; number of genes containing a peroxisome proliferator response element according to Lemay *et al*.

obvious distinction from the other donors. However, no difference could be found in the expression of *PPARα* between the donors at basal level, and also after incubation with WY14,643 no change in expression of *PPARα* was observed (data not shown). Another reason for the variation could be the difference in concentration of the PPARα ligands, i.e. fatty acids, and other nutritional factors present in the blood during donation. Blood donors are commonly advised to eat before donating blood. To investigate whether the nutritional status influences changes in gene expression, we incubated PBMCs of four volunteers, obtained after a meal and after an overnight fast, with WY14,643. Using QPCR, we determined the changes in PBMC gene expression of genes that showed either a low (*PDK4*, *SLC25A20*, *ACAA2*) or a high varia-

Gene Name	Gene Description	Entrez ID	Mean FC	p-value	PPRE	SLR A	SLR B	SLR C	SLR D	SLR E	SLR F
HAMP	Hepcidin precursor (Liver-expressed antimicrobial peptide)	57817	-1.40	0.00076	predicted						
PTX3	Pentraxin-related protein PTX3 precursor	5806	-1.31	0.00780	predicted						
KCTD12	BTB/POZ domain-containing protein KCTD12	115207	-1.33	0.00037	predicted						
ZEB2	Zinc finger E-box-binding homeobox 2	9839	-1.28	0.00794	predicted						
PAK1IP1	p21-activated protein kinase-interacting protein 1 (PAK1-interacting protein 1)	55003	-1.21	0.03585	predicted						
CCL23	Small inducible cytokine A23 precursor	6368	-1.20	0.01667	predicted						
CCL19	Small inducible cytokine A19 precursor	6363	-1.20	0.02586	predicted						
NR1H3	Oxysterols receptor LXR-alpha (Liver X receptor alpha)	10062	-1.23	0.00207	reported						
RRAS	Ras-related protein R-Ras precursor (p23)	6237	-1.16	0.01775	predicted						
FZD2	Frizzled-2 precursor	2535	-1.23	0.01046	predicted						
ATPAF1	ATP synthase mitochondrial F1 complex assembly factor 1 isoform 1 precursor	64756	-1.16	0.03081	predicted						
FTSJ2	Putative ribosomal RNA methyltransferase 2	29960	-1.16	0.01201	predicted						
TOB1	Tob1 protein (Transducer of erbB-2 1)	10140	-1.14	0.03696	predicted						
HspBP1	Hsp70-binding protein 1 (Heat shock protein-binding protein 1)	23640	-1.13	0.02131	predicted						
CD68	Macrosialin precursor (CD68 antigen)	968	-1.15	0.01269	predicted						
PAQR4	Progestin and adipoQ receptor family member 4	124222	-1.12	0.04328	predicted						
C14orf4	RING finger protein C14orf4	64207	-1.14	0.01352	predicted						
MYADM	Myeloid-associated differentiation marker	91663	-1.14	0.00947	predicted						
MRPL48	39S ribosomal protein L48, mitochondrial precursor	51642	-1.11	0.03522	predicted						
STAG3	Cohesin subunit SA-3	10734	-1.24	0.00449	predicted						
MARVELD1	MARVEL domain containing 1	83742	-1.18	0.01274	predicted						
PRRT2	proline-rich transmembrane protein 2	112476	-1.14	0.04240	predicted						
TBC1D9	TBC1 domain family, member 9 (with GRAM domain)	23158	-1.18	0.00424	predicted						
FAM35A	Protein FAM35A	54537	-1.13	0.04945	predicted						
NUP62	L-amino-acid oxidase precursor	259307	-1.10	0.03627	predicted						
WTAP	Wilms' tumor 1-associating protein	9589	-1.11	0.03427	predicted						
SGK2	Serine/threonine-protein kinase Sgk2	10110	-1.11	0.02416	predicted						
CCNY	Cyclin fold protein 1 (Cyclin box protein 1)	219771	-1.09	0.04023	predicted						
DLEU1	deleted in lymphocytic leukemia,1	10301	-1.10	0.03335	predicted						
PDF	Peptide deformylase, mitochondrial precursor	64146	-1.09	0.04462	predicted						
PDF	Peptide deformylase, mitochondrial precursor	84342	-1.09	0.04462	predicted						
IQCG	IQ domain-containing protein G	84223	1.10	0.03782	predicted						
GBA2	Non-lysosomal glucosylceramidase	57704	1.12	0.02249	predicted						
TGFBR2	TGF-beta receptor type-2 precursor	7048	1.13	0.02284	predicted						
SLC25A28	Mitoferrin-2 (Mitochondrial iron transporter 2)	81894	1.14	0.00979	predicted						
NISCH	nischarin	11188	1.17	0.00321	predicted						
FASTK	Fas-activated serine/threonine kinase	10922	1.12	0.02913	predicted						
POLD4	DNA polymerase subunit delta 4	57804	1.11	0.03927	predicted						
FGR	Proto-oncogene tyrosine-protein kinase FGR	2268	1.14	0.01256	predicted						
CLDN15	Mitochondrial fission 1 protein	24146	1.13	0.02469	predicted						
GPS2	G protein pathway suppressor 2	2874	1.17	0.01149	predicted						
TMEM43	Transmembrane protein 43	79188	1.16	0.04998	predicted						
TAPBP	Tapasin precursor	6892	1.18	0.03900	predicted						
GPR174	Probable G-protein coupled receptor 174.	84636	1.12	0.03679	predicted						
LINS1	lines homolog 1	55180	1.19	0.01190	predicted						
POLG2	DNA polymerase subunit gamma 2	11232	1.19	0.00451	predicted						
LOC168455	hypothetical protein LOC168455	168455	1.19	0.00392	predicted						
HIFPH4	Putative HIF-prolyl hydroxylase PH-4	54681	1.14	0.01885	predicted						
C1orf162	Uncharacterized protein C1orf162.	128346	1.19	0.00462	predicted						
NPHP4	Nephrocystin-4 (Nephroretinin).	261734	1.23	0.00317	predicted						
OTUD5	OTU domain-containing protein 5.	55593	1.11	0.01592	predicted						
ARIH2	Protein ariadne-2 homolog	10425	1.12	0.01439	predicted						
U2AF1L4	U2 small nuclear RNA auxiliary factor 1-like 4 isoform 2	199746	1.13	0.02068	predicted						
AKAP8L	A-kinase anchor protein 8-like (	26993	1.13	0.00936	predicted						
CYP27B1	25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial precursor	1594	1.15	0.00400	predicted						
CBY1	Protein Chibby	25776	1.18	0.00107	predicted						
PPARG	Peroxisome proliferator-activated receptor gamma	5468	1.20	0.00669	predicted						
MITF	Microphthalmia-associated transcription factor	4286	1.15	0.00643	predicted						
TXNIP	Thioredoxin-interacting protein	10628	1.15	0.00553	predicted						
KIAA0467	Uncharacterized protein KIAA0467	23334	1.18	0.00250	predicted						
CXCL16	Small inducible cytokine B16 precursor	58191	1.17	0.00739	predicted						
VMO1	Vitelline membrane outer layer protein 1 homolog precursor	284013	1.20	0.00200	predicted						
GPIBb	glycoprotein Ib beta polypeptideprecursor	2812	1.18	0.02852	predicted						
INSIG1	Insulin-induced gene 1 protein	3638	1.13	0.04718	non DR1						
SCML1	Sex comb on midleg-like protein 1	6322	1.13	0.04360	predicted						
ACRC	ACRC protein	93953	1.21	0.01719	predicted						
TLR4	Toll-like receptor 4 precursor	7099	1.23	0.00938	predicted						
C15orf17	Uncharacterized protein C15orf17	57184	1.16	0.02275	predicted						
ERRFI1	ERBB receptor feedback inhibitor 1	54206	1.19	0.00438	predicted						
ZMAT1	zinc finger, matrin type 1 isoform 1	84460	1.18	0.00535	predicted						
TESK2	Dual specificity testis-specific protein kinase 2	10420	1.20	0.00694	predicted						
AKT1S1	Proline-rich AKT1 substrate 1	84335	1.23	0.00185	predicted						
BACH1	Transcription regulator protein BACH1	571	1.19	0.00544	predicted						
G0S2	Putative lymphocyte G0/G1 switch protein 2	50486	1.25	0.00540	predicted						
MBOAT5	Membrane-bound O-acyltransferase domain-containing protein 5	10162	1.26	0.00282	predicted						
ABCA1	ATP-binding cassette sub-family A member 1	19	1.32	0.00030	reported						
CD36	Leukocyte differentiation antigen CD36	948	1.29	0.03259	predicted						
CYP1A1	Cytochrome P450 1A1	1543	1.26	0.02995	predicted						
CLCF1	Cardiotrophin-like cytokine factor 1 precursor	23529	1.15	0.04082	predicted						
CAPN3	Neutral alpha-glucosidase C	825	1.18	0.00492	predicted						
SMAD3	Mothers against decapentaplegic homolog 3	4088	1.23	0.00127	predicted						
MMP19	Matrix metalloproteinase-19 precursor	4327	1.22	0.01219	predicted						
CPT1B	Carnitine O-palmitoyltransferase I, muscle isoform	1375	1.23	0.00272	predicted						
ATHL1	acid trehalase-like 1	80162	1.27	0.00362	predicted						
AP1G2	Junctophilin-4 (Junctophilin-like 1 protein).	8906	1.19	0.00136	predicted						
USP52	PAB-dependent poly(A)-specific ribonuclease subunit 2	9924	1.27	0.00012	predicted						
TSPYL2	TSPY-like 2	64061	1.21	0.00472	predicted						
LOC54540	hypothetical protein LOC54540	54540	1.27	0.00049	predicted						
PLA2G4B	Cytosolic phospholipase A2 beta	8681	1.16	0.02102	predicted						
IL11RA	Interleukin-11 receptor alpha chain precursor	3590	1.19	0.02288	predicted						
SLC25A34	solute carrier family 25, member 34	284723	1.22	0.00967	predicted						
VEGFA	Vascular endothelial growth factor A precursor	7422	1.22	0.00807	predicted						
TANFKBH	T-cell activation NFKB-like protein	84807	1.29	0.00236	predicted						
TMEM185A	Transmembrane protein 185A	84548	1.25	0.01367	predicted						
NXF1	Nuclear RNA export factor 1	10482	1.35	0.00047	predicted						
CSAD	Cysteine sulfinic acid decarboxylase	51380	1.37	0.00238	predicted						
CYorf15B	lipopolysaccaride-specific response 5-like protein	84663	1.42	0.00046	predicted						
TPP1	Tripeptidyl-peptidase 1 precursor	1200	1.40	0.03811	predicted						
SFRS16	Splicing factor, arginine/serine-rich 16	11129	1.44	0.02157	predicted						
TMEM135	Transmembrane protein 135	65084	1.66	0.00142	predicted						
ACAA2	3-ketoacyl-CoA thiolase, mitochondrial (Acetyl-CoA acyltransferase)	10449	1.46	0.00002	predicted						
SLC25A20	Mitochondrial carnitine/acylcarnitine carrier protein (Carnitine/acylcarnitine translocase)	788	1.52	0.00000	reported						
ACADVL	Very-long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor	37	2.06	0.00000	predicted						
ADFP	Adipophilin (Adipose differentiation-related protein)	123	3.78	0.00000	reported						
PDK4	Pyruvate dehydrogenase kinase 4	5166	4.72	0.00000	reported						
FABP4	Fatty acid-binding protein, adipocyte	2167	13.89	0.01455	reported						

Figure 2

Genes changed after incubation with WY14,643, containing a predicted or reported PPRE. Heatmap of the signal log ratio of genes changed upon incubation with WY14,643 that contained a predicted or reported PPRE. Red indicates up-regulation compared to the vehicle incubated PBMCs and green indicates down-regulation. SL R, signal log ratio; PPRE, peroxisome proliferator response element

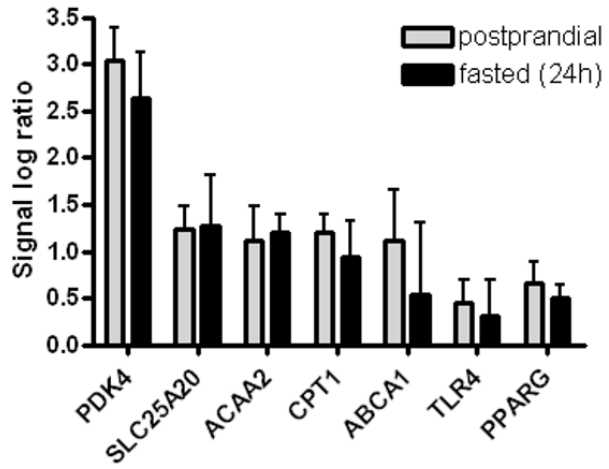

Figure 3
Gene expression changes of PBMCs incubated with WY14,643 isolated postprandial or after an overnight fast. Mean gene expression changes of PBMCs incubated with WY14,643, isolated postprandial or after an overnight fast. Error bars indicate standard deviations. *PDK4, Pyruvate dehydrogenase kinase 4; SLC25A20, carnitine-acylcarnitine translocase; ACAA2, acetyl-Coenzyme A acyltransferase 2; CPT1, Carnitine palmitoyltransferase 1; ABCA1, ATP binding cassette transporter 1; TLR4, Toll-like receptor-4; PPARγ, peroxisome proliferator-activated receptor gamma.*

tion (*CPT1, ABCA1, TLR4, PPARγ*) between donors in the microarray analyzes of the first study (Figure 3). No significant differences were observed in gene expression between the fasted and postprandial state.

To analyze whether the genes that did not have a PPRE according to Lemay *et al*. might have other transcriptional binding sites, a network analysis and a subsequent transcription factor binding site search with Genomatix software was performed. The network analysis showed that, besides the transcription factor PPAR, the transcription factors NFkB, JUN, TP53, SP1 and CTNNB1 were also directly linked to at least 10 genes from the list of 1,373 genes, The subsequent search for binding sites resulted in an additional 122 genes that could be linked to a PPRE and revealed that another 371 genes could be linked to at least one of the other selected transcription factors (see Additional file 1).

To obtain a selection of robust responding genes upon activation of PPARα with WY14.643, genes were selected that were more than 10% up or down regulated in all donors. This resulted in a list of 58 genes, including several known PPARα target genes (*ADFP, PDK4, SLC25A20*) (Figure 4), with a main function in fatty acid β-oxidation. Remarkably, only 16% of the genes in this list contained a predicted or reported PPRE.

To validate our data observed with microarray analyzes a selection of genes changed in the microarray analyzes was also measured with quantitative real time PCR (Q-PCR). In concordance with our microarray results, Q-PCR analyzes resulted in similar changes in expression of all genes analyzed (Figure 5).

PPARα regulation in PBMCs during fasting

Figure 6 shows the genes changed upon 24 hours fasting in healthy human volunteers with the number of genes that contain a PPRE. Comparison of gene expression profiles of PBMCs incubated with the PPARα ligand WY14,643 and fasted for 24 hours resulted in an overlap of 238 genes, indicating that around 14% of the genes changed during fasting are regulated by PPARα (Figure 7). Pathway analysis showed that these 238 genes were primarily involved in fatty acid metabolism. We found no overlap in pathways involved in amino acid metabolism. Exploration of the genes involved in fatty acid metabolism showed that fatty acid β-oxidation was specifically regulated, both in WY14,643 incubated cells and in PBMCs isolated after fasting (data not shown)

Discussion

In the present study, we showed that activation of the nuclear receptor PPARα in peripheral blood mononuclear cells results in a considerable change in gene expression profiles, as 10.5% of the genes expressed exhibited altered gene expression levels after incubation with the specific PPARα agonist WY14,643. The main function of PPARα in PBMCs appeared to be the regulation of fatty acid β-oxidation and other lipid metabolism related functions, which is in line with results from mice studies in liver [16] and intestine [17], and human cell line studies [18,19]. Moreover, the observed down-regulation of amino acid metabolism has been shown before in liver in studies comparing wild type mice to the PPARα knock out mouse model [7].

Besides the possible roles of PPARα in PBMCs, this study also demonstrates strong individual variability between the subjects in gene expression responses to activation with WY14,643. It appears that each donor has its own specific gene expression profile response to PPARα activation, which results in distinct differences in the expression of certain genes after WY14,643 incubation. Beck *et al*. also reported differences in responsiveness in gene expression between individuals, after incubation of endothelial cells with LPS. However, endothelial cell cultures were already divided beforehand into type I or type II responders based on their LPS mediated IL8 production [20]. In another study, incubation of cultured macrophages with

oxidized low-density lipoprotein resulted in a person-specific inflammatory gene expression response that could be correlated to changes in gene expression of scavenger receptors [21]. However, we did not find a correlation between basal *PPARα* expression or changes in *PPARα* expression and the observed variation in gene expression changes. In addition, the differences observed are probably not caused by the nutritional status of the subjects at baseline, as we did not observe differences in expression changes of selected PPARα target genes between the post-prandial and the fasted state of PBMCs incubated with WY14,643. However, it should be noted here that only four subjects were studied. A reason for the difference in response of the donors in the first study could be genetic variation, such as single nucleotide polymorphisms (SNPs) in the *PPARα* gene, its target genes or PPARα co-factors involved in activation of gene transcription. Furthermore, epi-genetic variation such as methylation status

Gene Name	Gene Description	Mean FC	*p* value	Entrez ID	PPRE	SLR A	SLR B	SLR C	SLR D	SLR E	SLR F
ADFP	Adipose differentiation-related protein	3.78	2.86E-10	123	reported						
PDK4	Pyruvate dehydrogenase kinase isozyme 4	4.72	6.16E-07	5166	reported						
ACADVL	Very-long-chain specific acyl-CoA dehydrogenase	2.06	1.05E-07	37	predicted						
LY6G5B	Casein kinase II subunit beta	1.88	4.82E-05	58496	-						
LOC283874	Hypothetical protein FLJ20393	1.66	8.72E-05	283874	-						
SLC25A20	Mitochondrial carnitine/acylcarnitine carrier protein	1.52	8.68E-07	788	reported						
IMPA2	Inositol monophosphatase 2	1.47	1.01E-05	3613	-						
C21orf7	TAK1-like protein.	1.40	1.79E-04	56911	-						
ST14	Suppressor of tumorigenicity protein 14	1.60	2.84E-05	6768	-						
HS3ST1	Heparan sulfate glucosamine 3-O-sulfotransferase 1	1.51	2.55E-04	9957	-						
ETFDH	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	1.42	1.32E-04	2110	-						
MICALL2	MICAL-like protein 2.	1.36	1.65E-04	79778	-						
MAP3K8	Mitogen-activated protein kinase kinase kinase 8	1.60	2.35E-04	1326	-						
TMEM135	Transmembrane protein 135.	1.66	1.42E-03	65084	predicted						
ACAA2	3-ketoacyl-CoA thiolase, mitochondrial	1.46	2.37E-05	10449	predicted						
BZRAP1	Peripheral-type benzodiazepine receptor-associated protein 1	1.36	2.93E-05	9256	-						
ABCC3	Canalicular multispecific organic anion transporter 2	1.34	9.88E-05	8714	-						
LOC158830	similar to Ab2-183	1.41	1.51E-04	158830	-						
C20orf119	Polyadenylate-binding protein 1-like.	1.40	2.80E-04	80336	-						
PPP1R3E	Homeobox and leucine zipper protein Homez	1.31	1.29E-04	90673	-						
PILRB	paired immunoglobulin-like type 2 receptor beta isoform b	1.32	1.58E-04	29990	-						
HBEGF	Heparin-binding EGF-like growth factor	1.36	7.70E-05	1839	-						
CXCL2	Macrophage inflammatory protein 2-alpha	1.31	6.44E-04	2920	-						
CCDC17	coiled-coil domain containing 17	1.25	6.35E-04	149483	-						
CYorf15B	lipopolysaccaride-specific response 5-like protein	1.42	4.61E-04	84663	predicted						
NLRC4	Caspase recruitment domain-containing protein 12	1.36	3.15E-04	58484	-						
USP52	PAB-dependent poly(A)-specific ribonuclease subunit 2	1.27	1.17E-04	9924	predicted						
FNBP4	formin binding protein 4	1.29	3.83E-04	23360	-						
COG3	Conserved oligomeric Golgi complex component 3	1.23	6.07E-04	83548	-						
CD300A	CMRF35-H antigen precursor	1.26	5.91E-04	11314	-						
ABCC5	Multidrug resistance-associated protein 5	1.24	9.97E-04	10057	-						
CREBZF	CREB/ATF bZIP transcription factor	1.26	2.51E-03	58487	-						
MPP7	palmitoylated membrane protein 7	1.24	8.44E-04	143098	-						
ECH1	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	1.23	1.38E-03	1891	-						
DEADC1	deaminase domain containing 1	1.20	5.78E-04	134637	-						
CLK2	Dual specificity protein kinase CLK2	1.19	1.52E-03	1196	-						
TTRAP	TRAF and TNF receptor-associated protein	1.23	1.15E-03	51567	-						
SPG7	Paraplegin	1.19	9.28E-04	6687	-						
MTX3	metaxin 3	1.14	5.25E-03	345778	-						
CCDC130	Coiled-coil domain-containing protein 130	1.20	1.18E-03	81576	-						
CBY1	Protein Chibby	1.18	1.07E-03	25776	predicted						
SYTL1	Synaptotagmin-like protein 1	1.17	1.75E-03	84958	-						
C6orf70	Uncharacterized protein C6orf70	1.17	2.19E-03	55780	-						
GPNMB	Transmembrane glycoprotein NMB	-1.41	9.23E-06	10457	-						
EMR1	EGF-like module-containing mucin-like hormone receptor-like 1	-1.38	1.30E-05	2015	-						
ANXA3	Annexin A3	-1.42	3.57E-05	306	-						
TMEM176A	Transmembrane protein 176A	-1.29	4.95E-05	55365	-						
CAPG	Macrophage-capping protein	-1.30	1.06E-04	822	-						
DPYSL2	Dihydropyrimidinase-related protein 2	-1.38	6.02E-05	1808	-						
IGSF6	immunoglobulin superfamily, member 6	-1.31	2.52E-04	10261	-						
IFI6	Interferon-induced protein 6-16	-1.32	3.05E-04	2537	-						
ADORA3	Adenosine A3 receptor	-1.34	8.81E-04	140	-						
C1orf115	Uncharacterized protein C1orf115.	-1.23	2.82E-04	79762	-						
TRIP10	Cdc42-interacting protein 4	-1.20	9.05E-04	9322	-						
FPR1	fMet-Leu-Phe receptor	-1.22	5.83E-04	2357	-						
TNFRSF8	Tumor necrosis factor receptor superfamily member 8	-1.18	2.37E-03	943	-						
GAS2L1	GAS2-like protein 1	-1.18	1.18E-03	10634	-						
C1orf163	Hcp beta-lactamase-like protein C1orf163.	-1.18	1.43E-03	65260	-						

Figure 4

Genes changed more than 10% in all individuals after incubation with WY14,643. Heat map of genes changed more than 10% in all individuals after incubation with WY14,643. Red indicates up-regulation and green indicates down-regulation. SLR, signal log ratio; PPRE, peroxisome proliferator response element

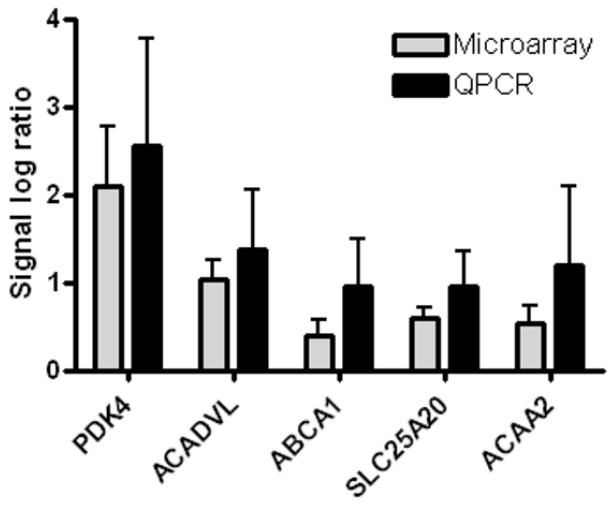

Figure 5
Comparison of microarray and quantitative real time PCR analysis. Mean gene expression changes of microarray and quantitative real time PCR analysis (Q-PCR) of six genes after incubation with WY14,643. Error bars indicated standard deviations. *PDK4, Pyruvate dehydrogenase kinase 4; ACADVL, acyl-Coenzyme A dehydrogenase, very long chain ; ABCA1, ATP binding cassette transporter 1; SLC25A20, carnitine-acylcarnitine translocase; ACAA2, acetyl-Coenzyme A acyltransferase 2*.

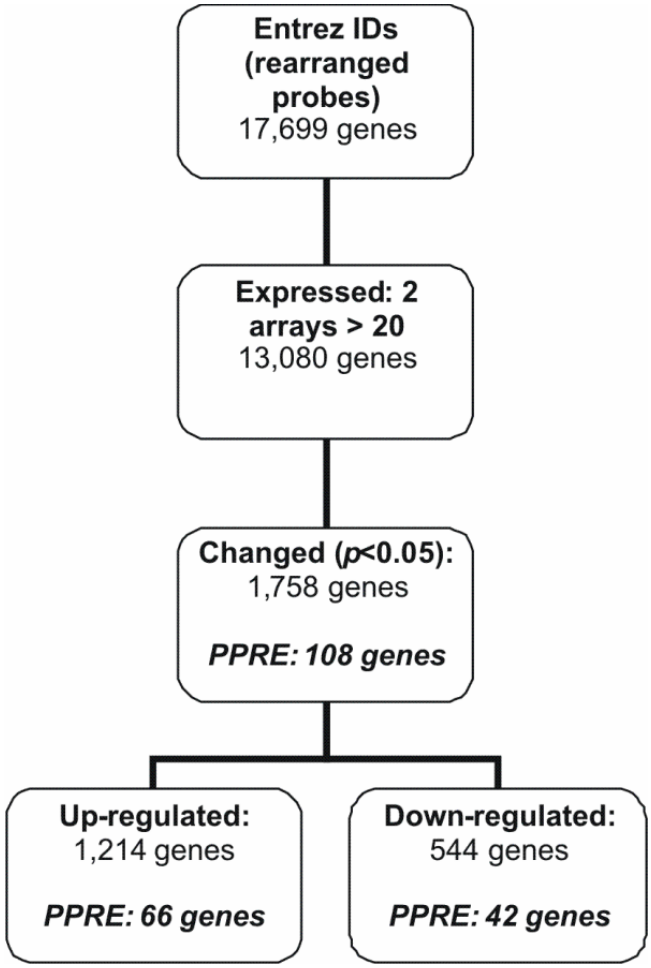

Figure 6
Gene selection procedure after microarray analysis of PBMC of three 24 hour fasted subjects. Flow chart of the followed gene selection procedure after microarray analysis of PBMC of three 24 hour fasted subjects. PPRE; number of genes containing a peroxisome proliferator response element according to Lemay *et al*. Data from this fasting study was published previously [14], but has been used here after applying a different annotation procedure.

of the PPARα promoter or its target genes may have caused between-subject differences in gene expression levels. Additional studies are required to elucidate whether gene expression profiles can be clustered in different response profiles, simplifying the identification of factors responsible for these individual responses. With respect to personalized nutrition these individual responses are of great interest as it can be expected that nutrients such as fatty acids can induce similar variations in response as WY14,643, which in the end might lead to personalized dietary advice.

The PPRE analyzes of the genes changed showed that approximately 8% of the genes changed after incubation with the PPARα ligand WY14,643 contained a predicted or reported PPRE, using the list as described by Lemay et al [15]. However, Lemay *et al*. report that they tolerate a low false-positive, and a high (60%) false-negative rate, suggesting that their list of PPREs is far from complete. Our additional transcription factor binding site analysis increase the number of genes that contain a PPRE to a total of 17% of the genes changed. A network search showed that, besides PPAR, five other transcription factors were involved in direct regulation of at least 10 out of the 1,373 changed genes. Interestingly, all these transcription factors are known to be affected by PPARα activation [22-26]. Transcription factor binding site analysis revealed that, out of the changed genes that did not contain a PPRE, 27% contained a binding site for at least one of the other five selected transcription factors These genes appear not to be regulated by PPARα directly, but indirectly, via these other transcription factors, a mechanism which has been suggested before [27,28]. The role of PPARα in this respect seems to be extensively larger than expected based on the outcome of PPRE analyzes alone.

An interesting observation is the decrease in expression level of genes containing a PPRE. Activation of PPARα by a ligand may result in a negative regulation of genes by

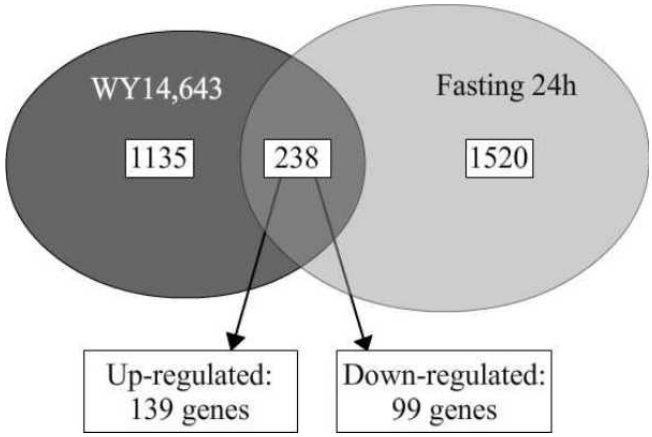

Figure 7
Overlap between genes changed upon WY14,643 incubation and after 24 hours fasting. Venn diagram of overlap between genes changed upon WY14,643 incubation and after 24 hours fasting.

means of transrepression as has been reported in several studies and reviewed by Ricote and Glass (2007) [29]. This transrepression, however, does not require the presence of PPREs in the promoter regions of the target genes. Apparently, negative regulation of these genes, regardless of its mechanism, is stronger than the transcriptional activation of PPARα. Previously, Degenhardt et al. (2006) also showed down regulation of an insulin-like growth factor-binding protein gene (IGFBP-6) that contained a predicted PPRE, in response to the presence of a PPARα ligand [30].

The overlap in gene expression profiles between fasting and incubation with WY14,643 shows that PPARα in PBMCs carries out a substantial part of its function during fasting, when concentrations of its natural ligands, free fatty acids, are elevated in the blood. The main role of PPARα in PBMCs during fasting is fatty acid β-oxidation, most likely to cope with the reduced availability of glucose for utilization in energy production and the increase of fatty acids.

Direct comparison between the two array analysis should be examined with care, since the two studies are distinctly different in set-up. The fasting intervention study was conducted in vivo, while the WY14,643 incubation experiments were performed ex vivo. Moreover, fasting involves many more changes in physiology, apart from the beforementioned increase in plasma free fatty acids, including changes in plasma insulin, glucose and leptin concentrations. The PPARα ligand incubations were set-up to elucidate the specific effects of activation of one nuclear factor, controlling for all other parameters.

Summarizing, this study gives us valuable information on the extent of the effect of PPARα activation, during fasting and in general, on human PBMC gene expression. It also shows that persons respond differently to PPARα activation with respect to their gene expression changes, indicating a possible person-specific nutrient response. It seems justified to conclude that human PBMCs are a suitable model to study changes in PPARα activation. This opens up the possibilities for more specific PPARα signaling studies in healthy humans using these relatively easily obtainable blood cells.

Methods

PBMC incubation

PBMCs from six healthy Caucasian male blood donors, aged between 30 and 48 yr, were isolated directly after arrival of the buffy coat (max. 8 hours after donation) by Ficol-paque Plus density gradient centrifugation (Amersham Biosciences, Roosendaal, the Netherlands). PBMCs were incubated in RPMI1640 medium with 2 mmol/L L-glutamine, 10% fetal bovine serum and antibiotics (penicillin and streptomycin) in the presence of 5% CO_2 at 37°C. at 1.0×10^6 cells per ml with either WY14,643 (50 μM) or vehicle (DMSO, 0.1%) for 12 hours. All donors gave full written informed consent.

Pre- vs. postprandial incubation

PBMCs of four healthy volunteers, aged between 28 and 34, were isolated after a meal and after an overnight fast. PBMCs were incubated at 1.0×10^6 cells per ml with either WY14,643 (50 μM) or vehicle (DMSO, 0.1%) for 12 hours. All volunteers gave full written informed consent.

Statistical methods

A 2-tailed paired *t* test was used to determine significant differences in Q-PCR gene expression values between the postprandial and the fasted state. Statistical significance was accepted at p 0.05. All calculations were performed with the use of the SPSS (version 12.0.1; SPSS, Chicago, IL).

Microarray processing

For 6 donors of the incubation experiments, total RNA from PBMCs was labeled using a one-cycle cDNA labeling kit (Affymetrix Inc, Santa Clara, CA) and hybridized to Affymetrix Human whole genome U133 plus 2.0 arrays (Affymetrix). Sample labeling, hybridization to chips and image scanning was performed according to the manufacturer's GeneChip Expression Analysis Technical Manual (Affymetrix).

Intervention study

For comparison of microarray data of the abovementioned incubation study, with microarray data of PBMC of fasted volunteers, we used the earlier described microarray

data of a 48 hours fasting study [14]. Briefly, four healthy male Caucasian volunteers, between 19 and 22 year of age were fasted for 48 hours. PBMCs were isolated out of blood taken at baseline, after 24 hours and after 48 hours of fasting. All volunteers gave full written informed consent and the study protocol was approved by the medical ethics committee of Wageningen University.

Microarray analysis

Microarrays were analyzed using the reorganized oligonucleotide probes as described by Dai *et al* (2005) [31]. Dai *et al*. combined all individual probes for a gene, enabling the possibility to detect the overall transcription activity of a gene, based on the latest genome and transcriptome information, instead of the Affymetrix probe set annotation. Application of this annotation procedure on the previously published data from the 48 hours fasting study [14] resulted in a difference in number of genes expressed and changed as compared to the previously used annotation method as this analysis was performed on probe set level.

Expression values were calculated using the Robust Multichip Average (RMA) method. RMA signal value estimates are based on a robust average of background corrected perfect match intensities and normalization was performed using quantile normalization [32,33]. Only genes with normalized signals higher then 20 on at least two out of twelve arrays were defined as expressed and selected for further analysis. Genes were defined as 'changed' when comparison of the normalized signal intensities showed a p-value lower then 0.05 in a two-tailed paired t test with Bayesian correction (Limma) [34]. Pathway analysis was performed using Ingenuity Pathway Analysis 5.5 (Ingenuity Systems). Array data have been submitted to the Gene Expression Omnibus, accession number GSE11289.

PPRE incidence

To indicated which of the genes changed upon activation of PPARα had a predicted or reported peroxisome proliferator response element (PPRE), we used information from Lemay *et al*. [15]. This paper recently reported predicted PPRE on a genome wide scale, using computational genomics and also summarized known PPRE. Using Genomatix software [35], network analysis was performed on 1,373 genes in BiblioSphere, from which transcription factors were selected that were directly linked to more than 10 genes from our list of changed genes. Subsequent transcription factor binding site analysis identified transcription factor binding sites in the promoters of our genes of interest that were cocited at least once in an abstract with these transcription factors. Heat maps were created by using Spotfire software.

cDNA synthesis and quantitative real-time PCR

RNA was reverse transcribed with the use of the cDNA synthesis kit (Promega, Leiden, the Netherlands). Standard quantitative real-time polymerase chain reaction (Q-PCR) was performed with the use of Platinum Taq DNA polymerase and SYBR Green on an iCycler PCR machine (Bio-Rad Laboratories BV) and duplicated at least twice. Primer sequences used in the PCRs were chosen based on the sequences available in PRIMERBANK [36]. Q-PCR data were normalized by measuring cycle threshold ratios between candidate genes and a housekeeping gene, human acidic ribosomal phosphoprotein PO, which was shown to be consistent within PBMCs [37].

Authors' contributions

MB collected and analyzed the data and wrote the manuscript. LAA an MM participated in critical revising of the manuscript. None of the authors has a personal or financial conflict of interest.

Additional material

Additional file 1

***Transcription factor binding site analysis**. Presence of transcription factor binding sites in the genes changed in PBMC after incubation with WY14,643. Transcription factors were selected if they directly affected at least 10 genes that were changed after WY14,643 incubation, in a network search using BiblioSphere (Genomatix). FC, fold change; PPRE (Lemay), peroxisome proliferator response element according to Lemay* et al.*[15]; NFkB, Nuclear factor kappa B binding site; JUN, Jun oncogene binding site; TP53, Tumor protein 53 binding site; SP1, Specificity protein 1 binding site; CTNNB1, catenin beta 1 binding site. Red indicates up regulated, green indicates down regulated*

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