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Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice

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de Wilde J, Mohren R, van den Berg S, Boekschoten M, Willems-Van Dijk K, de Groot P, Müller M, Mariman E, Smit E. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. *Physiol Genomics* 32: 360–369, 2008. First published November 27, 2007; doi:10.1152/physiolgenomics.00219.2007.—The prevalence of the metabolic syndrome (MS) is rapidly increasing all over the world. Consequently, there is an urgent need for more effective intervention strategies. Both animal and human studies indicate that lipid oversupply to skeletal muscle can result in insulin resistance, which is one of the characteristics of the MS. C57BL/6J mice were fed a low-fat (10 kcal%) palm oil diet or a high-fat (45 kcal%; HF) palm oil diet for 3 or 28 days. By combining transcriptomics with protein and lipid analyses we aimed to better understand the molecular events underlying the early onset of the MS. Short-term HF feeding led to altered expression levels of genes involved in a variety of biological processes including morphogenesis, energy metabolism, lipogenesis, and immune function. Protein analysis showed increased levels of the myosin heavy chain, slow fiber type protein, and the complexes I, II, III, IV, and V of the oxidative phosphorylation. Furthermore, we observed that the main mitochondrial membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, contained more saturated fatty acids. Altogether, these results point to a morphological as well as a metabolic adaptation by promoting a more oxidative fiber type. We hypothesize that after this early positive adaptation, a continued transcriptional downregulation of genes involved in oxidative phosphorylation will result in decreased oxidative capacity at a later stage. Together with increased saturation of phospholipids of the mitochondrial membrane this can result in decreased mitochondrial function, which is a hallmark observed in insulin resistance and Type 2 diabetes.

early onset of the metabolic syndrome; transcriptomics; nutrigenomics; muscular adaptation; fiber type conversion

THE METABOLIC SYNDROME (MS) is defined as a cluster of metabolic abnormalities including glucose intolerance (i.e., impaired glucose tolerance, Type 2 diabetes, or impaired fasting glycemia), abdominal obesity, dyslipidemia, and hypertension that are associated with insulin resistance (7, 14, 19, 27, 40, 59, 65). All these abnormalities are linked with an increased risk for developing cardiovascular diseases (7, 14, 19). Weight reduction is a way to manage the MS, because it improves all risk factors associated with the MS (14). Nevertheless, the prevalence of the MS is rapidly increasing all over the world. So, there is an urgent need for more effective intervention strategies (14, 65).

Skeletal muscle is an important tissue for insulin-stimulated glucose metabolism (1, 31, 40, 43). The switch to a high-fat (HF) diet will result in elevated levels of plasma triglycerides, resulting in increased fluxes of fatty acids (FA) into muscle tissue (49). A healthy skeletal muscle is characterized by the ability to switch easily between glucose and fat oxidation in response to these homeostatic signals and will promote the oxidation of FA as source for energy. This is known as metabolic flexibility (6, 48). The transcriptional coactivator, peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC1α), has been implicated in the regulation of energy metabolism. PGC1α induces mitochondrial biogenesis and increases expression of enzymes acting in fatty acid oxidation, the tricarboxylic acid cycle and oxidative phosphorylation (30, 47). PGC1α is expressed preferentially in muscle tissue enriched in type I fibers that contain a high concentration of mitochondria and use oxidative metabolism as energy source. It has been observed that muscle tissue of mice overexpressing PGC1α are greatly enriched in type I fibers, showing increased expression of genes involved in mitochondrial oxidative metabolism (e.g., the *cox2* and *cox4* genes) and muscle contraction (e.g., the *Tnni1* gene) (35, 46).

Another way in which skeletal muscle function can be affected is by lipid metabolites directly. Prolonged increased fluxes of FA into muscle tissue can result in the accumulation of FA as long-chain acyl CoAs (LC-CoAs), diacylglycerol (DAG), and triacylglycerol. There is evidence that LC-CoAs, DAG, or the lipid derivative ceramide interferes with the insulin signaling pathway (2, 8, 18, 20, 36). A strong relationship between impaired insulin-stimulated glucose metabolism and increased intramuscular lipid pools has been frequently described (1, 2, 16, 20, 37, 43, 45). Also a relation between insulin resistance and the FA composition of skeletal muscle membrane phospholipids has been demonstrated in both humans and animals. It was observed that the greater the percentage of polyunsaturated FA in membrane phospholipids, the better the insulin action (22, 41, 60).

We investigated the short-term effects of an HF palm oil diet on the mouse skeletal muscle. By performing genome-wide analysis of HF diet-responsive genes combined with protein and lipid analysis, we aimed for a better understanding of molecular events underlying the early onset of the MS. Eventually, this can contribute to the development of more effective nutritional intervention strategies.

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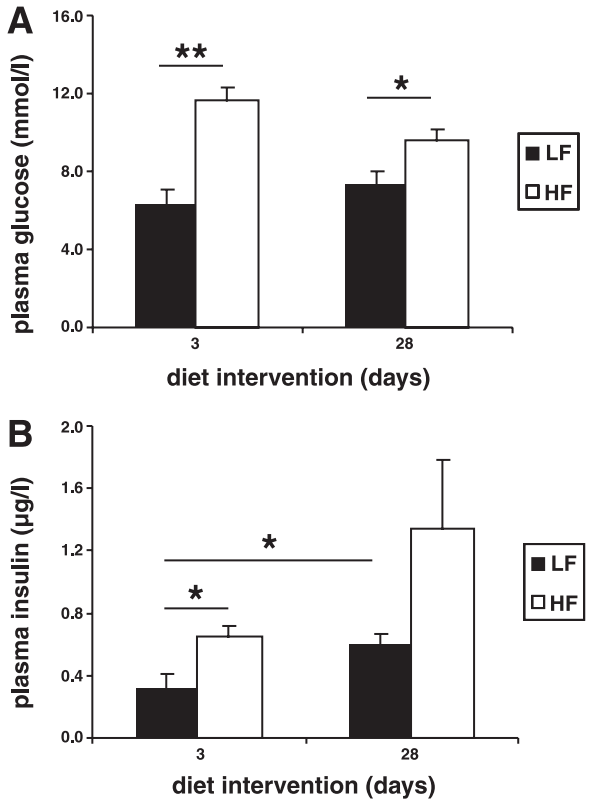

Fig. 1. Fasting blood glucose and insulin levels after an LF or an HF diet for 3 or 28 days. Values are means ± SE of the LF mice and the HF mice (n = 5 or n = 6). Significant differences: *$P < 0.05$ and **$P < 0.01$, respectively (obtained from independent samples t-test). LF, low fat; HF, high fat.

METHODS

Animals and study protocol. Male C57BL/6J mice were obtained from Charles River (Maastricht, The Netherlands) at 7 wk of age. The mice were fed a standard growth diet [RM3(E)DU; Special Diet Services, Witham, UK] for 2 wk prior to switching to the low-fat (LF) run-in diet for 3 wk. Following the run-in period mice were randomly assigned to the LF diet or the HF diet. The diets contained 10 kcal% fat or 45 kcal% fat in the form of palm oil (based on D12450B and D12451; Research Diet Services, Wijk bij Duurstede, the Netherlands). After 3 and 28 days mice were killed (n = 6 per group). Quadriceps muscles were dissected and snap-frozen in liquid nitrogen. Animal experiments were approved by the animal experimentation committee of Leiden University.

Measurements of fasting plasma glucose and fasting insulin levels. Fasting plasma glucose and insulin levels were measured using the glucose hexokinase assay (Roche Molecular Chemicals, Basel, Switzerland) and by ELISA (Mercodia, Uppsala, Sweden), respectively.

Affymetrix microarray analysis. Mouse total RNA was isolated from the quadriceps muscle with TRIzol reagent (Invitrogen, Breda, the Netherlands) and purified with the RNeasy Mini Kit (Qiagen, Venlo, the Netherlands). Contaminating genomic DNA was removed with the RNase-free DNase set (Qiagen). RNA quantity was measured with the ND-1000 spectrophotometer (Isogen Life Science, IJsselstein, the Netherlands), and RNA integrity was checked on an Agilent 2100 BioAnalyzer (Agilent Technologies, Amsterdam, The Netherlands) using nanochips according to the manufacturer's instructions. Affymetrix GeneChip Mouse Genome 430 2.0 arrays were used to determine the mouse quadriceps muscle transcriptome during LF diet and HF diet intervention. Individual microarrays were performed for each sample. Ten micrograms of RNA were used for one cycle cRNA synthesis (Affymetrix, Santa Clara, CA). Hybridization, washing, and scanning of Affymetrix Mouse Genome 430 2.0 arrays were done according to standard Affymetrix protocols. Array images were processed using packages from the Bioconductor project (17). Probe sets were redefined according to Dai et al. (11). In this method probes are annotated using up-to-date databases and assigned to unique gene identifiers, in this case Entrez IDs, instead of the "classic" GeneChip probe sets. This results in a less ambiguous and more accurate annotation. Arrays were normalized using quantile normalization, and expression estimates were calculated using GC robust multiarray average background adjustment implementing the empirical Bayes estimate for nonspecific binding (63). Differentially expressed probe sets were identified using linear models, applying moderated t-statistics that implement empirical Bayes regularization of standard errors (53). Comparison was between the LF mice and the HF mice. Probe sets that satisfied the criterion of a P value <0.05 and fold-change >1.3 were considered to be significantly regulated. To relate changes in gene expression to functional changes two complementary methods were used. The first method is based on overrepresentation of Gene Ontology (GO) terms, which uses a gene score resampling method (33). Full resampling was run with 200,000 iterations. Only classes with a false discovery rate (FDR) <0.001 and with minimal 8 and maximal 125 genes were taken into account. The second method, gene set enrichment analysis (GSEA), is focused on predefined gene sets, that is, groups of genes that share biological function, chromosomal location or regulation (55). The "functional catalogue" constructed by Subramanian et al. (55) was modified to contain only 505 well-defined murine, biochemical, metabolic, and signal pathways compiled from the following publicly available, curated databases: BioCarta (BioCarta, 2005, http://www.biocarta.com), GenMAPP (10), Kyoto Encyclopedia of Genes and Genomes (KEGG) (28), Sigma-Aldrich pathways (Sigma-Aldrich Metabolic and Cell Signaling pathways, 2005), and Signal Transduction Knowledge Environment (STKE; 2005, http://stke.sciencemag.org/). The analysis was run using 1,000 permutations per gene set. Gene sets with an FDR <0.05 were considered as significantly regulated. The advantage is that both methods are unbiased and a score is computed based on all genes in a GO term or gene set. Array data have been submitted to the Gene Expression Omnibus, accession number GSE8524.

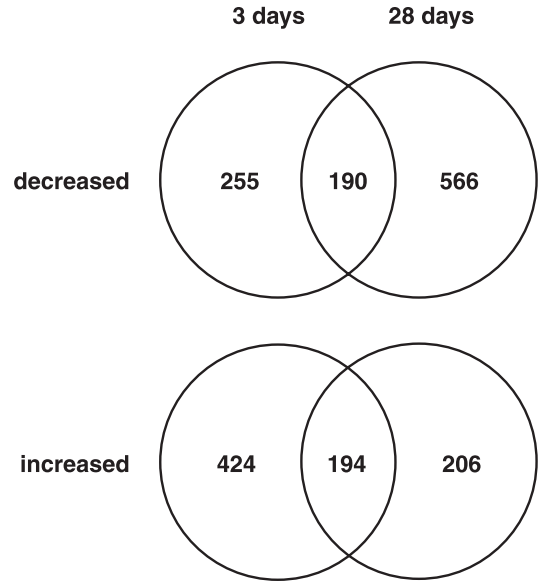

Fig. 2. Venn diagrams of the number of genes with a decreased and increased expression after 3 days and 28 days of HF diet intervention, respectively. Genes that satisfied the criteria of fold change >1.3 and P value <0.05 were considered significantly regulated.

Real-time quantitative PCR. To validate microarray-detected changes real-time quantitative PCR (qPCR) was performed using individual cDNA samples. RNA (1 μg) was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Veenendaal, the Netherlands). Primers were designed across exon boundaries when possible using Beacon Designer5 (Bio-Rad). The qPCR reactions were performed in a volume of 25 μl containing 12.5 ng cDNA, 1× IQ SYBR Green Supermix (Bio-Rad) and 400 nM of gene-specific forward and reverse primers (Biolegio, Nijmegen, the Netherlands). cDNA was amplified using a two-step program (40 cycles of 10 s at 95°C and 45 s at 60°C) with a MyiQ system (Bio-Rad). Specificity of amplification was verified by melt curve analysis and evaluation of efficiency of PCR amplification. Gene expression levels were determined using qBase analysis software (23) using a Δ-Ct relative quantification model with PCR efficiency correction and multiple reference gene normalization. The geometric mean of three internal control genes, the *Canx*, *Hprt1*, and *Arbp* genes, was calculated using Genorm (56) and used as a normalization factor in the qBase analysis software (23).

Table 1. *Top 25 of overrepresented Gene Ontology classes after 3 days and 28 days of HF feeding*

GO ID	GO Class	*N*	Raw Score	FDR
	Day 3			
GO:0006937	regulation of muscle contraction	24	1.48	3.86E-04
GO:0014033	neural crest cell differentiation	25	1.39	6.04E-10
GO:0048762	mesenchymal cell differentiation	30	1.32	2.01E-04
GO:0014031	mesenchymal cell development	29	1.30	4.03E-04
GO:0006936	muscle contraction	63	1.27	2.42E-10
GO:0009855	determination of bilateral symmetry	29	1.21	8.91E-04
GO:0008366	axon ensheathment	29	1.21	9.21E-04
GO:0008064	regulation of actin polymerization and/or depolymerization	38	1.20	3.94E-04
GO:0030832	regulation of actin filament length	39	1.18	2.16E-04
GO:0051258	protein polymerization	41	1.16	1.10E-10
GO:0043122	regulation of I-κB kinase/NF-κB cascade	50	1.13	1.51E-10
GO:0008154	actin polymerization and/or depolymerization	48	1.12	1.95E-04
GO:0051128	regulation of cell organization and biogenesis	61	1.05	2.08E-04
GO:0007179	transforming growth factor beta receptor signaling pathway	45	1.05	6.97E-04
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	46	1.05	8.63E-04
GO:0006818	hydrogen transport	66	1.04	5.25E-11
GO:0006119	oxidative phosphorylation	49	1.03	2.32E-04
GO:0051270	regulation of cell motility	63	1.01	7.55E-11
GO:0008360	regulation of cell shape	45	1.01	7.83E-04
GO:0015992	proton transport	60	1.00	1.78E-04
GO:0007187	G-protein signaling, coupled to cyclic nucleotide second messenger	45	1.00	7.69E-04
GO:0030334	regulation of cell migration	56	0.99	1.83E-04
GO:0040012	regulation of locomotion	66	0.99	1.73E-10
GO:0051789	response to protein stimulus	58	0.99	2.42E-04
GO:0031589	cell-substrate adhesion	66	0.98	1.21E-09
	Day 28			
GO:0050729	positive regulation of inflammatory response	11	3.10	3.90E-11
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	14	3.06	1.75E-11
GO:0006957	complement activation, alternative pathway	10	2.77	3.78E-04
GO:0048002	antigen processing and presentation of peptide antigen	28	2.71	9.29E-11
GO:0002478	antigen processing and presentation of exogenous peptide antigen	17	2.60	2.42E-11
GO:0002438	acute inflammatory response to antigenic stimulus	10	2.57	7.55E-04
GO:0002697	regulation of immune effector process	9	2.53	5.75E-04
GO:0046460	neutral lipid biosynthesis	9	2.52	6.93E-04
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	15	2.48	1.68E-11
GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	16	2.36	2.20E-04
GO:0019884	antigen processing and presentation of exogenous antigen	22	2.29	2.63E-11
GO:0050766	positive regulation of phagocytosis	14	2.27	1.17E-04
GO:0006635	fatty acid beta-oxidation	13	2.26	1.15E-04
GO:0002822	regulation of adaptive immune response (sensu Gnathostomata)	11	2.25	4.85E-04
GO:0006094	gluconeogenesis	18	2.17	6.50E-05
GO:0050727	regulation of inflammatory response	24	2.14	1.55E-11
GO:0006641	triacylglycerol metabolism	11	2.13	6.58E-04
GO:0002541	activation of plasma proteins during acute inflammatory response	32	2.09	3.10E-11
GO:0019882	antigen processing and presentation	47	2.06	1.66E-11
GO:0001523	retinoid metabolism	14	2.05	8.37E-04
GO:0006639	acylglycerol metabolism	16	2.03	4.54E-04
GO:0006637	acyl-CoA metabolism	13	2.01	4.51E-04
GO:0006739	NADP metabolism	13	2.00	4.44E-04
GO:0046486	glycerolipid metabolism	17	1.98	7.55E-05
GO:0006090	pyruvate metabolism	23	1.97	2.47E-11

A scoring-based resampling method was used to identify significantly overrepresented GO classes in the skeletal muscle of the 3 days high-fat (HF) mice and the 28 days HF mice, respectively. The analysis was performed using the tool ErmineJ (33). For the concept biological processes we selected 25 Gene Ontology (GO) classes with a false discovery rate (FDR) <0.001 and with the highest raw score. For this analysis only classes containing 8 through 125 genes were taken into account. *N*, number of genes in GO class.

Protein analysis by Western blotting. Individual mouse muscle samples were crushed under liquid nitrogen and homogenized in lysis buffer containing 10% (wt/vol) SDS, 5 mM DTT, 20 mM Tris base, and proteinase inhibitor cocktail tablets (Roche Molecular Chemicals, Basel, Switzerland). After centrifugation protein samples at 14,000 *g* for 20 min at 10°C, supernatant was aliquoted and stored at −80°C. Protein concentration was measured with a BCA protein assay kit (Pierce, Etten-Leur, the Netherlands). Total protein was separated by SDS-PAGE on 4–15% Criterion gels (Bio-Rad) at 150 V and transferred to a polyvinylidene fluoride membrane (Bio-Rad) for 90 min at 100 V. Blocking and antibody (Ab) incubation steps of the membrane were performed in TBST (10 mM Tris·HCl, 100 mM NaCl, 0.1% Tween 20, pH 7.6) supplemented with 5% nonfat dry milk (Bio-Rad). Membranes were incubated overnight with anti-mouse polyclonal Pgc1α Ab (1:1,000; Chemicon, Amsterdam, the Netherlands), anti-mouse polyclonal Fas Ab (1:350; Abcam, Cambridge, UK), anti-mouse monoclonal sterol regulatory element binding protein 1 (Srebp1) Ab (1:200; Santa Cruz Biotechnology, Heidelberg, Germany), anti-mouse polyclonal Scd1 Ab (1:200, Santa Cruz Biotechnology), anti-mouse monoclonal oxidative phosphorylation (OXPHOS) Ab cocktail directed to the ND6 subunit of complex I, the 30 kDa Ip subunit of complex II, the 47 kDa core protein 2 of complex III, subunit IV of complex IV, and the α-subunit of F_1F_{10} ATP synthase (complex V) (1:2,000; Mitosciences, Eugene, OR) or anti-mouse monoclonal myosin (skeletal, slow) Ab (1:10,000 Sigma, Zwijndrecht, the Netherlands) at 4°C. After washing with TBST, membranes were incubated with a HRP-conjugated secondary Ab (Dako, Glostrup, Denmark). Signals were detected by ECL using Pierce reagents and CL-Xpose clear blue X-ray film (Perbio Science, Etten-Leur, the Netherlands). Gel images were obtained with a GS-800

Table 2. *Changed gene sets after 3 days and 28 days of HF feeding*

	Day 3				*Day 28*			
	N	ES	NES	FDR	N	ES	NES	FDR
Upregulated Cellular Processes								
Striated muscle contraction[1]	41	0.73	2.57	<0.001	41	0.72	2.43	<0.001
Cell communication[2]	91	0.57	2.30	<0.001	nc			
Mitochondrial fatty acid oxidation[3]	29	0.71	2.26	<0.001	29	0.69	2.16	<0.001
Ecm receptor interaction[2]	81	0.57	2.24	<0.001	nc			
Electron transport chain[1]	63	0.60	2.23	<0.001	nc			
Oxidative phosphorylation[2]	97	0.56	2.22	<0.001	nc			
Mitochondrial fatty acid beta oxidation[1]	15	0.76	2.10	0.001	nc			
Tissues blood and lymph[1]	31	0.63	2.08	0.002	nc			
Fatty acid beta oxidation[1]	34	0.60	1.95	0.008	nc			
Ribosome[2]	21	0.65	1.93	0.008	nc			
Ribosomal proteins[1]	29	0.61	1.94	0.009	nc			
Focal adhesion[2]	171	0.44	1.91	0.010	nc			
Fatty acid oxidation[1]	29	0.58	1.88	0.014	nc			
Smooth muscle contraction[1]	152	0.41	1.76	0.047	nc			
Downregulated Cellular Processes								
Proteasome[2]	25	−0.70	−2.27	<0.001	25	−0.59	−1.79	0.047
Aminoacyl-tRNA-biosynthesis[2]	24	−0.64	−2.07	0.007	nc			
Amino acid metabolism[1]	46	−0.55	−2.01	0.010	46	−0.64	−2.20	0.001
Proteaome degradation[1]	60	−0.49	−1.92	0.023	nc			
Lipogenesis[3]	nc				28	−0.79	−2.38	<0.001
Adipogenesis[3]	nc				126	−0.56	−2.27	<0.001
Antigen processing and presentation[2]	nc				52	−0.61	−2.16	0.001
Pentose phosphate pathway[2]	nc				25	−0.71	−2.13	0.002
Galactose metabolism[2]	nc				32	−0.65	−2.03	0.005
Ppar signaling pathway[2]	nc				64	−0.57	−2.04	0.005
Lipoprotein metabolism[3]	nc				31	−0.65	−2.05	0.005
Starch and sucrose metabolism[2]	nc				50	−0.60	−2.06	0.005
Porphyrin and chlorophyll metabolism[2]	nc				22	−0.66	−1.93	0.015
Complement and coagulation pathways[1]	nc				58	−0.51	−1.89	0.023
Fatty acid biosynthesis[1]	nc				23	−0.64	−1.87	0.026
Statin pathway[1]	nc				20	−0.67	−1.87	0.029
Bisphenol A degradation[2]	nc				16	−0.67	−1.86	0.029
Metabolism of xenobiotics by cytochrome P450[2]	nc				50	−0.53	−1.85	0.030
Apoptosis[1]	nc				43	−0.53	−1.82	0.041
Glycan structures degradation[2]	nc				24	−0.60	−1.79	0.044
Nucleotide sugars degradaion[2]	nc				16	−0.67	−1.78	0.045
Oxidative stress[1]	nc				25	−0.60	−1.79	0.046
Ascorbate and aldarate metabolism[2]	nc				15	−0.67	−1.78	0.046
Insulin signaling pathway[2]	nc				122	−0.43	−1.77	0.046
Type I diabetes mellitus[2]	nc				38	−0.54	−1.77	0.047
Nitrogen metabolism[2]	nc				21	−0.61	−1.76	0.048

Gene set enrichment analysis was applied to identify upregulated and downregulated processes after 3 days and 28 days of HF feeding. The analyses were performed as described (30). Presented are regulated processes with an FDR <0.05. An FDR was calculated to adjust for multiple hypothesis testing. Sources of the gene sets: [1]GenMAPP, [2]KEGG, [3]SKmanual. N, number of genes; ES, enrichment score for the gene set that reflects the degree to which a gene set is overrepresented at the top or bottom of the ranked list; nc, not changed; NES, normalized enrichment score, i.e., the normalized ES to account for the size of the set.

calibrated densitometer and analyzed with Quantity One (Bio-Rad). Since the Gapdh protein was stably expressed under the dietary conditions we applied, we have used the Gapdh signal to calculate the relative protein abundance.

Lipid metabolome analysis. The composition of lipids in the quadriceps muscle of mice fed an LF or an HF diet for 28 days ($n = 3$ per group) was determined by high-throughput methods developed by Lipomics (San Diego, CA). Briefly, lipids from the quadriceps muscle (20–40 mg) were extracted with chloroform-methanol (2:1, vol/vol) in the presence of authentic internal standards by the method of Folch et al. (15). Individual lipid classes within the extract were separated by preparative thin-layer chromatography. Subsequently, FA methyl esters were separated and quantified as described previously Watkins et al. (61).

Statistical analyses. Unless otherwise indicated, all analyses were performed with SPSS for Windows, version 13.0. Means were compared per time point with an independent samples *t*-test, and a *P* value <0.05 was considered statistically significant.

RESULTS

Physiological effects of the HF diet. After 3 days or 28 days of diet intervention, neither total body weight nor the weight gain for the HF diet group was significantly different from that of the LF diet group (data not shown). However, plasma glucose levels were significantly increased after 3 days and 28 days of HF feeding. Also the plasma insulin levels were already significantly increased after 3 days of HF diet and were still higher at 28 days of diet intervention (Fig. 1). The increase in glucose and insulin levels at 3 days was also observed in other experiments and may be induced by the change in diet.

HF diet-induced changes in the skeletal muscle transcriptome. Microarray analysis using Affymetrix whole genome mouse arrays revealed that the expression of numerous genes was significantly changed after 3 days and 28 days of HF diet intervention. When using the criteria of a fold change >1.3 and a *P* value <0.05, we found that the expression level of 1,063 genes was significantly different between the 3 days LF mice and the HF mice. Of these 1,063 genes, 445 genes were expressed at a lower level and 618 genes were expressed at a higher level in the HF mice compared with the LF mice. The expression level of 400 genes was increased and the expression level of 756 genes was decreased in the 28-day HF mice compared with the LF mice. The expression of 384 genes was altered to the same direction in both the 3-day HF mice and the 28-day HF mice (Fig. 2). A complete list of regulated genes is available in Supplement 1.[1] qPCR analyses were performed for 16 genes validating the microarray results (Supplement 2).

To gain insight into the biological processes affected by the HF diet, a scoring-based resampling method was applied to identify significantly overrepresented GO classes (33). The top 25 of gene classes with respect to the concept Biological Process that changed most significantly are listed in Table 1. After 3 days of diet intervention, the top 25 of overrepresented GO classes consisted mainly of descriptors for morphogenesis like muscle contraction, cell differentiation, and tissue development. Other overrepresented GO classes included descriptors for fatty acid catabolism and immune system. After 28 days of diet intervention, the top 25 of overrepresented GO classes were mainly descriptors of immune system, inflammation, and energy metabolism. A complete list of overrepresented GO classes is available in Supplement 3. A parallel GSEA was used to focus on groups of genes that share biological function or regulation (55). This method allows the identification of up- or downregulated processes making it a more refined method than GO-based analysis. However, due to overlap in the source databases several processes are represented multiple times. GSEA revealed that numerous gene sets were upregulated after 3 days of HF feeding. Almost all increased gene sets were related to FA catabolism and muscle contraction. Only four gene sets were downregulated and were related to protein degradation and amino acid metabolism. Twenty-eight days of HF feeding resulted in the upregulation of two gene sets representing striated muscle contraction and mitochondrial FA oxidation. The downregulated gene sets corresponded to many diverse cellular processes such as carbohydrate catabolism, detoxification, immune function, lipogenesis, and lipoprotein metabolism (Table 2). The outcome of the GSEA had numerous similarities to the outcome of the GO-based analysis. Both methods resulted in the identification of descriptors for FA metabolism and morphogenesis. However, the outcome of the GSEA also had some differences with the outcome of the GO-based analysis. For example, GSEA resulted in numerous downregulated gene sets describing lipogenesis, whereas descriptors for this metabolic process were not present in the top 25 of overrepresented GO classes. This demonstrates that these methods should not be used separately but in a complementary way. The functional outcomes of the GO-based analysis and GSEA are summarized in Fig. 3.

Western blotting analysis revealed an adaptation to the HF diet. Three of the outcomes of the transcriptome analyses were that the HF diet suppressed lipogenesis and enhanced morphogenesis and FA catabolism, respectively. To look into this at the protein level, we analyzed the abundance of myosin heavy chain, slow fiber type protein (morphogenesis), Pgc1α protein, and subunits of the five complexes of the oxidative phosphorylation (FA catabolism), and of the Srebp1, Scd1, and Fas proteins (lipogenesis) after 28 days of HF feeding (Fig. 4).

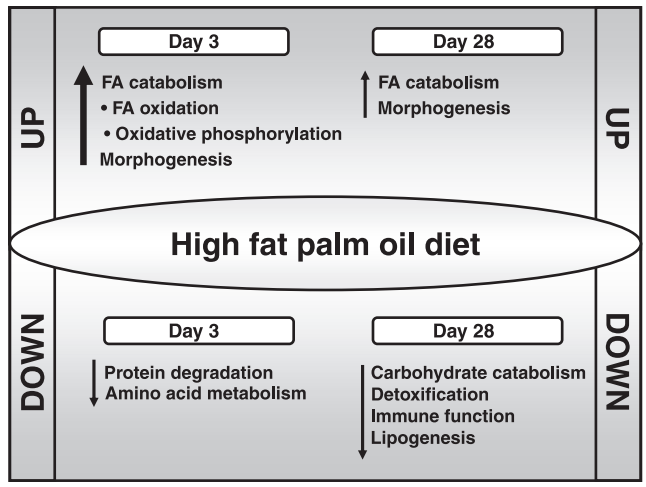

Fig. 3. An HF palm oil diet altered expression of genes in a variety of biological processes in mouse skeletal muscle. Summary of the functional implications of an HF palm oil diet as assessed by analyses of predefined gene sets based on Gene Ontology, biochemical, metabolic, and signal pathways. FA, fatty acid.

[1] The online version of this article contains supplemental material.

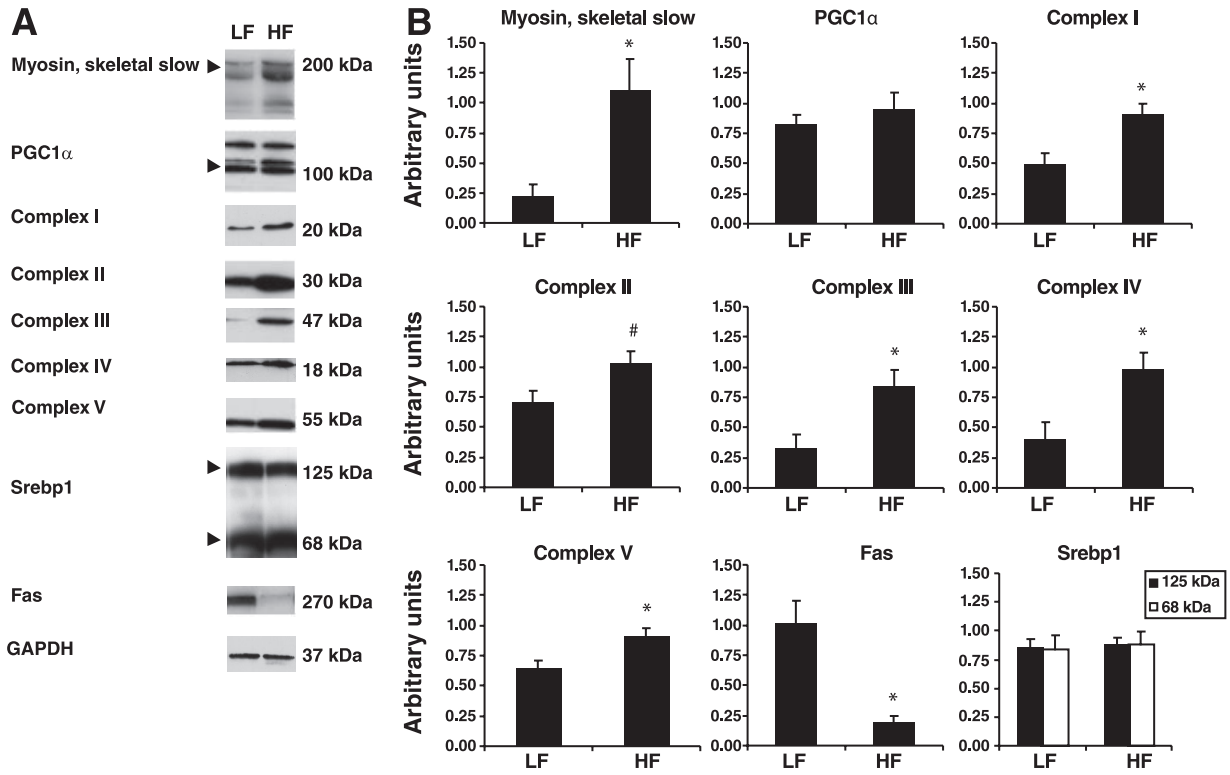

Fig. 4. Western blotting analyses of various proteins in quadriceps muscle. *A*: representative examples of Western blotting of equal amounts of total quadriceps muscle protein of the 28-day LF mouse ($n = 5$) and 28-day HF mouse ($n = 4$). Arrowheads indicate the quantified protein. *B*: quantified protein expression levels are means + SE of 5 (LF) or 4 (HF) biological replicates; #Tendency toward significant difference with $P < 0.10$; *significant difference with $P < 0.05$ (obtained from independent samples *t*-test). The Gapdh protein signal was used for normalization. PGC, peroxisome proliferator-activated receptor-γ coactivator; Srebp, sterol regulatory element binding protein.

Western blotting showed that the myosin heavy chain, slow fiber type protein content was increased by 5.1-fold in the quadriceps muscle of the HF mice, confirming the increased gene expression of markers for type I fibers (*Tnni1*, 15.3-fold; *Tnnc1*, 12.6-fold; *Tnnt1*, 12.3-fold; *Myh7*, 11.4-fold). Although we observed a 1.4-fold increased expression of the *Ppargc1a* gene, no differences were found in the Pgc1α protein content. GSEA showed an upregulation of the gene set describing mitochondrial FA oxidation. Western blotting revealed that the protein abundance of five oxidative phosphorylation subunits was increased (complex I, 1.9-fold $P = 0.02$; complex II, 1.5-fold $P = 0.05$; complex III, 2.6-fold $P = 0.03$; complex IV, 2.5-fold $P = 0.04$; and complex V, 1.4-fold $P = 0.04$) in the HF mice compared with the LF mice. The HF diet did not influence the abundance of the Srebp1 protein, confirming the unchanged *Srebf* gene expression. Also the processing of the Srebp1 protein, as judged by the ratio of the 125 kDa and the 68 kDa bands, was not changed by the HF diet. The expression of the *Scd1* gene was downregulated 16.0-fold. Despite the relatively high gene expression, the Scd1 protein

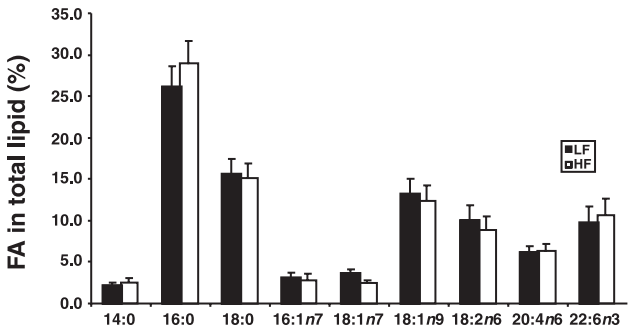

Fig. 5. FA composition of the mouse muscle lipid metabolome. FA with an abundance >2% were considered to be present. The most abundant FA are mystric acid (14:0) palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n7), vaccenic acid (18:1n7), oleic acid (18:1n9), linoleic acid (18:2n6), arachidonic acid (20:4n6), and docosahexaenoic acid (22:6n3). The FA composition of the mouse muscle lipid metabolome was found to be comparable between mice fed the LF and the HF diet. Values are means + SE of 3 biological replicates.

Table 3. *Effect of a 28-day HF diet on total fatty acids per lipid class*

Total FA/Lipid Class	LF	HF
Cholesterol ester	673±156	676±220
Diacylglycerol	276±23	260±29
Free fatty acid	1,034±196	1,015±133
Triacylglycerol	2,701±1,227	3,895±388
Cardiolipin	1,225±112	1,108±110
Lysophosphatidylcholine	401±28	310±33
Phosphatidylcholine	8,915±632	7,483±351
Phosphatidylethanolamine	5,692±887	4,137±186
Phosphatidylserine	963±37	903±99
Free Cholesterol	2,383±470	1,865±99

Values are expressed as nmol of total fatty acid per gram of muscle tissue and are means ± SE of 3 biological replicates. FA, fatty acid; LF, low-fat diet; HF, high-fat diet.

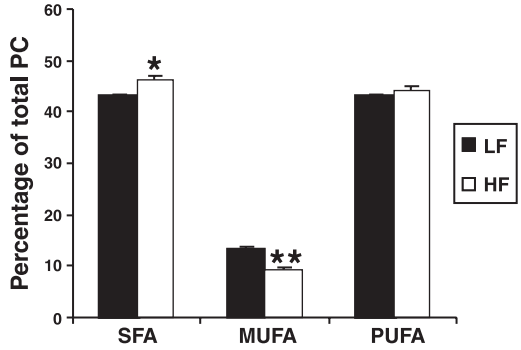

Fig. 6. Relative percentages SFA, MUFA, and PUFA in phosphatidylcholine. Values are means + SE of 3 biological replicates, Significant difference with $*P < 0.05$ and $**P < 0.01$, respectively (obtained from independent samples *t*-test). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

could not be detected in the mouse quadriceps muscle (data not shown). After 28 days of HF feeding a 5.6-fold decreased abundance of the Fas protein was detected, which is in line with the downregulation of the expression of the *Fasn* gene (53.2-fold).

Changes in the quadriceps muscle lipid metabolome are lipid class specific. Because of the observed gene and protein changes we expected effects on lipid content and lipid composition in the skeletal muscle. Therefore, we performed a lipid metabolome analysis. The most abundant FA in the quadriceps muscle lipid metabolome were palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n7), vaccenic acid (18:1n7), oleic acid (18:1n9), linoleic acid (18:2n6), arachidonic acid (20:4n6), and docosahexaenoic acid (22:6n3). Overall, the fatty acid composition of the quadriceps muscle lipid metabolome was found to be comparable between the LF and the HF mice ($n = 3$ per group) (Fig. 5). Also no significant differences were observed on the total level of individual lipid classes (Table 3). However, in the most predominant phospholipid class, phosphatidylcholine (PC) increased saturated fatty acids (SFA) and decreased monounsaturated fatty acids (MUFA) were observed for the HF mice compared with the LF mice (Fig. 6). When looking in more detail, we found that 16:0 was significantly increased not only in PC, but also in the phosphatidylethanolamine (PE) lipid class. Furthermore, 18:0 was decreased in the lipid classes PC and lysophosphatidylcholine (LYPC). The MUFA 16:1n7 and 18:1n7 were decreased in LYPC, PE, and PC, whereas 18:1n9 was decreased only in the phospholipid class PC (table 4).

DISCUSSION

The increasing prevalence of the MS in Western societies is caused by a combination of sedentary lifestyles and an increased consumption of diets rich in SFA. Evidence from both animal and human studies indicates that lipid oversupply to skeletal muscle results in functional perturbations that eventually result in impaired insulin-stimulated glucose metabolism, which is one of the characteristics of the MS (32, 36, 50). A better understanding of molecular mechanisms involved in the early onset of the MS can contribute to the development of more effective intervention strategies. Therefore we set out to investigate the short-term effects of a HF diet on the mouse muscle transcriptome by performing a genome-wide analysis of HF diet-responsive genes combined with protein and lipid analyses. Short-term HF feeding resulted in a changed expression of genes involved in a variety of biological processes including morphogenesis, energy metabolism, lipogenesis, and immune function.

The skeletal muscle contains myofibers that differ in contractile function, mitochondrial content, and metabolic properties. Slow-twitch (type I) fibers contain a high number of mitochondria and use oxidative metabolism as an energy source, whereas fast-twitch (type II) fibers generate energy mainly through glycolysis. The quadriceps muscle contains many type II fibers resulting in a low expression of the *Tnni1* gene, which is a classical marker of type I fibers (35). Short-term HF feeding resulted in enhanced expression of the *Tnni1* gene and other markers for type I fibers, such as the *Tnnt1*, *Tnnc1*, *Myl2,* and *Myh7* genes. In addition, the protein abundance of the myosin heavy chain, slow fiber type protein content was increased. Although the adult skeletal muscle is known to have the capacity to adapt to functional demands including exercise, innervation, mechanical loading, and hormones by a conversion of fiber type (44, 64), the link between HF feeding and fiber type conversion is yet unknown. Our findings indicate that the HF diet induces rapid morphological adaptations in the skeletal muscle of adult mice by inducing a conversion to a more type I fiber phenotype via a transcriptional mechanism. This may result in a more oxidative character of the quadriceps muscle. Recently, it was shown that PGC1α is a principal factor in regulating muscle fiber type determination (35, 46). Furthermore, both human and animal

Table 4. *Effect of 28-day HF diet on specific fatty acids per phospholipid class*

	LYPC			PC			PE		
	LF	HF	*P*	LF	HF	*P*	LF	HF	*P*
16:0	37.6±2.2	42.6±0.6		36.9±0.5	41.2±0.9	*	9.3±0.5	11.3±0.3	*
18:0	20.5±0.6	16.8±0.5	*	5.1±0.3	4.0±0.2	*	23.3±1.7	21.3±0.6	
16:1n7	1.7±0.7	1.0±0.1		2.6±0.2	1.3±0.2	*	0.5±0.0	0.3±0.0	†
18:1n7	1.9±0.2	1.3±0.1	*	4.0±0.1	2.4±0.3	†	0.9±0.1	0.3±0.1	*
18:1n9	7.5±0.8	8.0±0.4		6.5±0.1	5.3±0.1	†	4.2±0.6	5.1±1.0	
18:2n6	9.9±5.0	4.7±0.3		8.2±1.1	6.4±1.1		3.1±0.2	2.7±0.2	
20:4n6	5.4±0.6	5.2±0.3		15.5±0.3	15.4±9.9		11.0±0.2	10.3±0.3	

Values are expressed as FA mol percent (moles of an FA as a percentage of total mol of FA per lipid class) and are means ± SE of 3 biological replicates, Significant differences $*P < 0.05$ and $†P < 0.01$, respectively (obtained from independent samples *t*-test). LYPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

studies have associated Type 2 diabetes and insulin resistance with reduced expression of the *Ppargc1a* gene and genes involved in OXPHOS in muscle tissue (32, 42, 54). Although we showed that short-term HF feeding increased expression of the *Ppargc1a* gene, the PGC1α protein content was not changed. Despite this observation, protein analysis revealed a higher abundance of the OXPHOS complexes. This was also found on the mRNA level. Interestingly, the transcriptional upregulation was much more pronounced on *day 3* than on *day 28*. This observation indicates that over time OXPHOS gene expression tends to decrease while the protein levels are maintained. In view of recent studies (32, 42, 54) we hypothesize that this trend will continue, gradually resulting in decreased protein levels, which will result in a decreased oxidative capacity in the long run.

HF feeding resulted in a suppression of lipogenic genes such as the *Acaca*, *Acly*, *Elovl6, Fasn,* and *Scd1* genes. This was supported by analysis of the FAS protein. A transcription factor involved in lipogenesis is Srebp1 (13, 24, 25). Srebp1 is strongly expressed in lipogenic tissues, such as adipose tissue and liver. Recent studies have shown that Srebp1 is also expressed in skeletal muscle (9, 34). Srebp1 is synthesized as an inactive precursor bound to the endoplasmatic reticulum (ER). Upon activation, the ER-bound Srebp1 precursor undergoes a cleavage followed by translocation to the nucleus (13, 24). Neither the abundance nor the processing of the Srebp1 protein showed diet-dependent differences. A diet-dependent effect on the nuclear translocation remains possible. Alternatively, downregulation of lipogenic genes by other transcription factors cannot be excluded.

Our study connects HF feeding with the immune system of the skeletal muscle. Skeletal muscle cells can act as antigen presenting cells by expressing different major histocompatibility complex molecules, thus making skeletal muscle cells active participants in the T-cell immune response (38, 62). It is known that fat can reduce several parameters of the immune system such as antigen presentation, resulting in suppression of immune function (12, 21, 57, 58). Several studies have shown that activation of PPARα results in reduction of the immune function (5, 26, 39). Since PPARα was clearly activated in our study, as is evidenced by the consistent upregulation of a number of PPARα target genes, we hypothesize that also in the skeletal muscle the fat-induced suppression of the immune system may be mediated by PPARα.

The major components of the mitochondrial membrane are phospholipids. Membranes permit the maintenance and regulation of ionic gradients, potential differences, and the passage of hormones, substrates, nutrients, and intracellular signals, all of which are critical to normal cellular function (40, 41). As such, the fatty acyl composition and the degree of saturation of the membranes are of great importance for membrane fluidity and membrane permeability and thus for normal membrane function (4, 40, 59). We observed that HF feeding leads to a higher degree of saturation of PC and PE. Different studies have provided evidence linking obesity and insulin resistance to the FA composition of skeletal muscle membrane phospholipids (3, 41, 60). We suggest that a more saturated PC and a more saturated PE, which are the main mitochondrial membrane phospholipids (52), will make the mitochondrial membrane more rigid and less responsive (20). This may gradually diminish the function of for example, the membrane-bound OXPHOS subunits. In the long run, the disruption of the mitochondrial membrane may result in morphological aberrations like mitochondrial damage and reduced mitochondrial size, which has been observed in insulin resistance and Type 2 diabetes patients (29, 51, 54).

When looking for early molecular responses to the HF palm oil diet in the skeletal muscle of the mouse, we observed that short-term HF feeding resulted in a morphological adaptation by increasing genes and proteins that are markers for the more oxidative type I fibers. Simultaneously, our results point to a metabolic adaptation by increasing proteins involved in oxidative phosphorylation. We hypothesize that after early adaptation of the skeletal muscle a continued downregulation of genes involved in oxidative phosphorylation will eventually result in decreased protein abundance and oxidative capacity. Together with increased saturation of mitochondrial membrane phospholipids this can result in a reduced mitochondrial function that has been observed in insulin resistance and Type 2 diabetes. In conclusion, our results contribute to a better understanding of the molecular events underlying the early onset of the MS. This may benefit the development of more effective nutritional intervention strategies.

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GRANTS

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