Defining high-fat-diet rat models: metabolic and molecular effects of different fat types

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Abstract

High-fat (HF)-diet rodent models have contributed significantly to the analysis of the pathophysiology of the insulin resistance syndrome, but their phenotype varies distinctly between different studies. Here, we have systematically compared the metabolic and molecular effects of different HF with varying fatty acid compositions. Male Wistar rats were fed HF diets (42% energy; fat sources: HF-L – lard; HF-O – olive oil; HF-C – coconut fat; HF-F – fish oil). Weight, food intake, whole-body insulin tolerance and plasma parameters of glucose and lipid metabolism were measured during a 12-week diet course. Liver histologies and hepatic gene expression profiles, using Affymetrix GeneChips, were obtained. HF-L and HF-O fed rats showed the most pronounced obesity and insulin resistance; insulin sensitivity in HF-C and HF-F was close to normal. Plasma ω-3 polyunsaturated fatty acid (ω-3-PUFA) and saturated fatty acid (C_{12}-C_{14}, SFA) levels were elevated in HF-F and HF-C animals respectively. The liver histologies showed hepatic steatosis in HF-L, HF-O and HF-C without major inflammation. Hepatic SREBP1c-dependent genes were upregulated in these diets, whereas PPARα-dependent genes were predominantly upregulated in HF-F fed rats. We detected classical HF effects only in diets based on lard and olive oil (mainly long-chain, saturated (LC-SFA) and monounsaturated fatty acids (MUFA)). PUFA- or MC-SFA-rich diets did not induce insulin resistance. Diets based on LC-SFA and MUFA induced hepatic steatosis with SREBP1c activation. This points to an intact transcriptional hepatic insulin effect despite resistance to insulin's metabolic actions.

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Introduction

The coincidence of obesity, insulin resistance, hypertension and dyslipidemia is commonly referred to as the 'metabolic syndrome'. This condition affects approximately 20–40% of the population in the industrialized nations, and its prevalence is expected to rise further in the next decades (Laaksonen *et al.* 2004). Central obesity and alterations of adipokine secretion, together with a concomitant fat accumulation in different metabolically active tissues such as liver, muscle and pancreas, build the pathophysiologic basis of the metabolic syndrome (McPherson & Jones 2003, Unger 2003, Carr *et al.* 2004), and hepatic steatosis is now often added to the classical components mentioned above (Brunt 2004, den Boer *et al.* 2004). It is generally agreed that individual genetic background and lifestyle factors contribute to the pathogenesis of this disorder. Both nutrition and physical activity are major factors in determining its manifestation, but the exact chain of causation remains unclear.

Several rodent models have been used to study the pathogenesis of the metabolic syndrome. In view of the polygenic character of this disorder, monogenic models of obesity and diabetes, such as the ob/ob mouse or the obese Zucker (fa/fa) rat, do not reflect the human disease sufficiently. Models of acquired obesity generated by pharmacologic measures, such as the gold-thioglucose mouse model, certainly are unphysiologic in many respects. From this point of view, experiments with these obesity models will clarify only certain aspects of the metabolic syndrome and contribute little to the overall understanding of this condition's pathophysiology.

The first description of a 'high-fat diet' to induce obesity by a nutritional intervention was in 1959 (Masek & Fabry 1959). Subsequent studies have revealed that high-fat diets promote hyperglycemia and whole-body insulin resistance, and numerous researchers have examined their effects on muscle and liver physiology as well as insulin signal transduction. From this experience, it is generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised β-cell function (Oakes *et al.* 1997, Ahren *et al.* 1999, Lingohr *et al.* 2002).

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As evident from the literature, various diets with very different fatty acid compositions are summarized under the term 'high-fat diet'. This has inevitably led to considerable variability in the results reported. Most studies have employed only one high-fat formula in contrast with standard chow and did not analyze the influence of the specific fat component in the model. From the sparse data comparing different high-fat diets with respect to their metabolic effects, it is generally believed that diets based on saturated fatty acids induce the typical high-fat-diet phenotype, whereas diets containing polyunsaturated ω-3 fatty acids exert beneficial effects on body composition and insulin action (Storlien *et al.* 1991, 1996). Surprisingly, the role of monounsaturated fatty acids in this context remains to be defined.

A direct comparison of high-fat diets based on the main fatty acid subtypes with respect to morphometric and physiologic differences as well as gene expression changes has not been performed yet. Therefore, we have characterized and compared alterations induced by high-fat diets based on 1. coconut fat (saturated fatty acids (SFA)); 2. olive oil (monounsaturated fatty acids (MUFA)); 3. lard (comparable quantities of SFA and MUFA) and 4. fish oil (polyunsaturated fatty acids (PUFA)). This analysis included not only the obese phenotype and the degree of insulin resistance, but also changes in plasma lipid profiles, major hormones of metabolism and hepatic lipid deposition, as well as the gene expression pattern in the liver.

Materials and methods

Experimental animals

Six-week-old, male Wistar rats were purchased from Charles River (Sulzfeld, Germany). Rats were singly caged with free access to water and subjected to different dietary regimens as described below. Diets were prepared in pellet form by Altromin (Lage, Germany). Animals were kept on a 12:12-h light–darkness cycle. All animal procedures were approved by the local animal rights committee and complied with the German Law on Animal Protection as well as the UFAW 'Handbook on the care and management of laboratory animals', 1999.

Experimental design

After three days of acclimatization, the rats had free access to either a standard rodent chow (SC, fat content 11% of energy), or a high-fat diet (fat content 42% of energy), based on lard (HF-L), olive oil (HF-O), coconut fat (HF-C) or fish oil (derived from cod liver, HF-F). Weight gain and food intake were monitored once a week. After 12 weeks, an insulin tolerance test (see below) was undertaken. Two days later, the animals were killed after an overnight fast (16 h). Venous blood was drawn from the heart into EDTA-coated vials, and plasma was prepared and stored at –20 °C pending further analysis. Pancreatic islets were isolated as described below. Liver tissue samples were collected in 10% formaldehyde for histologic analysis or clamp-frozen in liquid nitrogen for lipid and mRNA analysis as described below. Unless otherwise stated, all reagents were purchased from Sigma or Merck at the highest purity grade available.

Insulin tolerance test

Experiments were performed with all rats in the high-fat diet and standard chow groups. Food was withdrawn for 20 h. Fasting glucose levels were then measured repeatedly for at least 30 min with a hand-held glucometer (AccuTrend; Roche) from whole blood drawn from the tail-tip capillary region. After establishment of a stable baseline glucose level, the animals were injected intraperitoneally with 0·15 U/kg body weight insulin (Aventis, Frankfurt, Germany). Whole-blood glucose levels were then monitored every 10 min for 30 min.

Liver histology and liver lipid profile

For histologic examination, liver pieces from the right ventral lobe were fixed in 10% neutral buffered formaldehyde, embedded in paraffin, cut, mounted on slides and stained according to standard hematoxylin-eosin protocols. Stained slides were analyzed by a board-certified pathologist (M W) in a blinded fashion. Tissue triglycerides were determined as described previously with slight modifications (Buettner *et al.* 2000). In brief, frozen liver samples were first powdered under liquid nitrogen. An amount of 20–50 mg frozen liver powder was then weighed into 1 ml chloroform–methanol mix (2:1 v/v) and incubated for 1 h at room temperature with occasional shaking to extract the lipid. After addition of 200 µl H_2O, vortexing and centrifugation for 5 min at 3000 ***g***, the lower lipid phase was collected and dried at room temperature. The lipid pellet was redissolved in 60 µl tert-butanol and 40 µl Triton X-114-methanol (2:1 v/v) mix, and triglycerides were measured with the GPO-triglyceride kit (Sigma) by appropriate triglyceride standards (Sigma).

Liver mRNA expression analysis

The two animals in each experimental group showing the most typical phenotype (as judged by weight gain, plasma parameters and insulin tolerance test) were chosen for liver mRNA expression analysis of important metabolic genes. Total RNA was isolated from clamp-frozen liver pieces with RNeasy mini-spin columns (Qiagen). The purified RNA was routinely checked for visible signs of degradation in a Bioanalyzer

2100 (Agilent Technologies, Palo Alto, USA), and only high-quality RNA was processed. Sample processing and data acquisition were carried out by the array facility of the University of Regensburg (Kompetenzzentrum Fluoreszente Bioanalytik). Biotinylated cRNA was prepared according to the recommended Affymetrix protocol (*GeneChip Expression Analysis Technical Manual*, Rev. 6, Affymetrix, Santa Clara, CA, USA). Briefly, 3–5 μg RNA were used to generate double-stranded cDNA (One-Cycle cDNA Synthesis Kit, Affymetrix), and biotinylated cRNA was synthesized in an *in vitro* transcription reaction (IVT Labeling Kit, Affymetrix). Fragmented cRNA was hybridized to Affymetrix Rat Genome 230 2·0 Arrays (16 h, 45 °C) in a rotating chamber. Arrays were washed and stained in a fluidic station, and scanned with a GeneChip Scanner 3000 (Affymetrix). Image processing and probe set level data analysis were performed with Affymetrix GCOS 1·1 software. Arrays were linearly scaled to a target value of 100 to ensure comparability between arrays. To assess array quality, several parameters were taken into account: background level and distribution, noise, mean signal intensity and ratio of signal levels for probe sets representing the 5′- and 3′-ends of actin and glyceraldehyde-3-phosphate dehydrogenase transcripts. Empirical cutoff values were defined, and samples that did not meet the defined criteria were discarded from further analysis to ensure comparability of arrays within the sample set. All array procedures complied with the Minimum Information About Microarray Experiments (MIAME) standard; the original data were submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), accession no. GSE3512.

As the mRNA expression in two animals from each diet group was separately analyzed, four comparisons were possible when comparing high-fat-diet animals with controls receiving standard chow. Genes fulfilling the Affymetrix quality criteria for significant expression were considered to be differentially expressed between the diet groups when the expression levels were concordantly increased or decreased in all four comparisons, or when all of the following criteria were met:

(1) The expression of the gene was significant in a specific diet group in both animals examined.

(2) Two out of the four comparisons were significantly increased or decreased, based on the algorithms of the Affymetrix software.

(3) The mean fold change was at least two-fold and the fold change of each individual comparison was at least 1·5.

For this study, only genes related to glucose and fat metabolism were further analyzed; gene clustering was performed with web-based analysis software (EASE/DAVID).

Real-time RT–PCR

Confirmation of microarray results was performed by real-time RT–PCR on independently derived RNAs (different rats) for all diet groups. Total RNA was isolated as described above, and RT–PCR was performed as described in detail elsewhere (Bollheimer *et al.* 2002). In brief, first-strand complementary cDNA was synthesized from equal amounts of total RNA by priming with arbitrary hexamers. For subsequent PCR amplification (standard RT–PCR and LightCycler system; Roche), the following primer pairs (1 μM) were employed:

1. 5′-ggagcaaatggccaaactaa-3′ (sense)/5′-tccctcaaatatg ccttgg-3′ (antisense) for enoyl-CoA-hydratase
2. 5′-tggcttccgttcagtctctt-3′ (sense)/5′-cagtgccaaggtctc tagcc-3′ (antisense) for fatty acid synthase
3. 5′-atgaccctgccaagaatgac-3′ (sense)/5′-tcccagggtaacg ctaacac-3′ (antisense) for very long chain acetyl-CoA-dehydrogenase
4. 5′-cagtggagcgtgaagacaaa-3′ (sense)/5′-cttggtccaattg aggagga-3′ (antisense) for glucokinase cDNA
5. 5′-tcacacaatgcaatccgttt-3′ (sense)/5′-ggccttgaccttgtt catgt-3′ (antisense) for PPARα cDNA
6. 5′-atgctgaagaggaagcctga-3′ (sense)/5′-gaagtccaggtg gccataga-3′ (antisense) for glycogen synthase cDNA
7. 5′-aggatgaggcctatgac-3′ (sense)/5′-cgtaggcttagctacc gta-3′ (antisense) for SREBP1c cDNA
8. 5′-ttgcgcttaaagctatagg-3′ (sense)/5′-gtccaaaggaatatg acacg-3′ (antisense) for 18s rRNA.

First-strand cDNA-samples were then amplified for 40 cycles (95 °C for 5 s, 60 °C for 5 s, 72 °C for 22 s). After verification of the RT–PCR product by gel electrophoresis, a LightCycler analysis was performed with the same temperature protocol. The formation of primer dimers was ruled out in all LightCycler experiments by melting curve analysis. The cDNA content for a specific gene in each sample was semiquantitatively assessed by comparing the experimentally determined crossing point with the crossing points and respective concentrations of a pooled standard cDNA, as described previously (Bollheimer *et al.* 2003). All results were normalized by the 18s-rRNA content to ensure comparability.

Free fatty acid concentration and free fatty acid profile

The plasma concentration of free fatty acids was determined with a commercially available kit (Wako-Chemicals, Neuss, Germany). The plasma free fatty acid profile was determined by HPLC, as previously described (Shimomura *et al.* 1986). In brief, free fatty acids were extracted by the addition of chloroform and vigorous shaking. After evaporation of chloroform, the residue was dissolved in methanol, mixed with 9-anthryl-diazo-methane and derivatized at

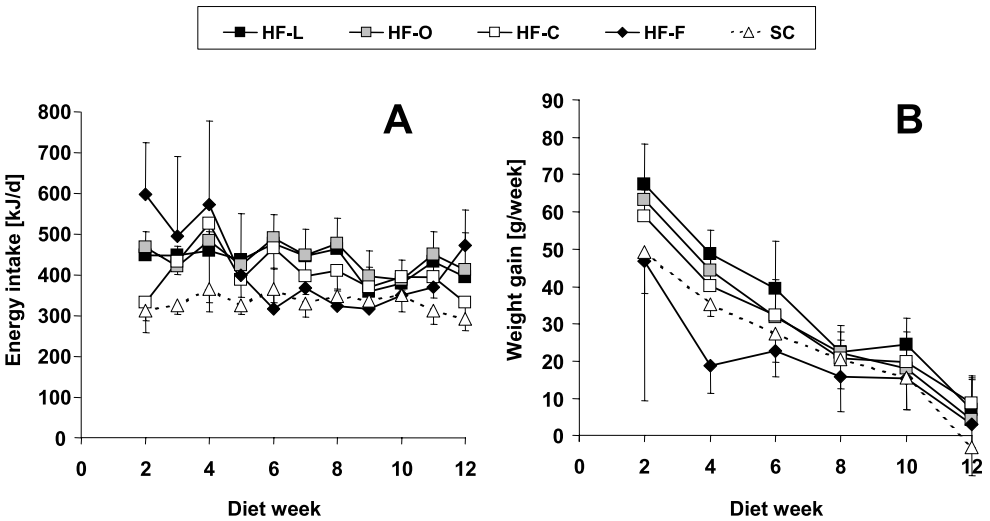

Figure 1 Energy intake (A) and weight gain (B) per week in Wistar rats fed different high-fat diets during the 12-week diet course. Given are the means±S.D. of 12 rats per diet group. Black boxes: lard-based, high-fat diet (HF-L); gray boxes: olive oil-based, high-fat diet (HF-O); white boxes: coconut fat-based, high-fat diet; black rhomboids: fish oil-based, high-fat diet (HF-F); white triangles: standard rodent chow (SC).

room temperature for 3 h. The derivatized fatty acids were then separated with Kontron HPLC with methanol/water as eluents. The HPLC system uses two pre-columns and two main columns. The separation of fatty acids is based on the different lengths of the free fatty acid and the different degree of saturation. Long-chain fatty acids and saturated fatty acids are retained longer in the column than short-chain fatty acids or unsaturated fatty acids. Internal standards are used for identification and quantification.

Insulin secretion analysis from pancreatic islets *ex vivo*

Pancreatic islets were isolated separately from individual animals treated with the different diet types by collagenase digestion and Histopaque-Ficoll density gradient centrifugation, as previously described (Bollheimer *et al.* 2003). Batches of 10 islets were placed into 150 µl Krebs-Ringer bicarbonate buffer, 0·1% (w/v) fatty acid-free BSA, 5·6 mM glucose and 16 mM Hepes (pH 7·4). The samples were incubated at 37 °C in a 95% CO_2 atmosphere. After 75 min and 90 min, 50 µl supernatant were removed and stored at –80 °C pending analysis for insulin.

Biochemical measurements

Plasma glucose, triglycerides, creatinine and alanine aminotransferase (AAT), as well as aspartate aminotransferase (AST) activities, were measured in the central laboratory of the University Hospital Institute of Clinical Chemistry by routine procedures. Insulin, glucagon and adiponectin were measured with rat-specific ELISA kits (Mercodia, Uppsala, Sweden; Linco Research, St Charles, MO, USA). The HOMA index (homeostasis model assessment) was calculated as follows: HOMA index=glucose (mmol/l)insulin (pmol/l)/155 (Matthews *et al.* 1985).

Statistical methods

To obtain representative data, all experiments were performed on 6–12 animals. Data are presented as means ± S.D Group differences was analyzed with an exact Fisher–Pitman permutation test (for $n \leq 6$) or unpaired Student's *t*-test (for $n>6$); numerical data were correlated with SPSS 12·0 statistics software (Chicago, IL, USA). The significance level was set to $P<0{\cdot}05$.

Results

Energy intake

The energy intake per week is shown in Fig. 1A for all diet groups; the cumulative energy intake is given in Table 1. On average, animals fed standard rodent chow (SC) consumed 322–343 kJ per day (95% CI) during the 12-week diet period, amounting to a mean total energy uptake of 25·8 MJ. The food intake in rats receiving high-fat diets based on lard (HF-L), olive oil (HF-O) or coconut fat (HF-C) was significantly higher at

Table 1 Basal characteristics of the dietary groups after 12 weeks. The values represent the means±S.D. of six independent experiments

	HF-L	HF-O	HF-C	HF-F	SC
Final weight (g)	606±54*,¶,#	577±54*,#	551±40*,#	428±56*	504±36
Cumulative food intake (MJ)	33±2*,#	33±3*,#	30±3*	27±5	26±2
Liver weight (g)	20·8±3·1*	21·3±3·5*	19·5±4·3	19·9±4·0	17·3±2·6
(% of body weight)	3·4±0·5#	3·7±0·4#	3·5±0·6#	4·7±0·4*	3·5±0·4#
Liver triglyceride (mg/g)	15·8±5·5*,¶,#	14·8±3·7*,¶,#	11·4±3·7*,#	5·0±2·0	4·0±2·4
Plasma characteristics					
Glucose (mmol/l)	5·4±0·7	5·6±0·3*,¶	5·0±0·5	5·0±1·1	5·0±0·3
Triglycerides (mmol/l)	2·1±1·0	2·4±1·3	2·8±1·5*,#	1·1±0·7	1·2±0·4
Free fatty acids (µmol/l)	0·63±0·19	0·72±0·16#	0·72±0·20	0·51±0·11	0·53±0·16
Creatinine (µmol/l)	26·5±2·7	25·6±3·5	25·6±3·5	23·0±3·5	25·6±2·7
AST (IU/l)	119±59	127±38	140±68	87±31	124±37
ALT (IU/l)	48±3#	57±19	46±5*,#	64±6	71±25
Insulin (pmol/l)	780±230*,#	654±270	762±277	539±318	577±223
Glucagon (nmol/l)	0·81±0·09*	0·72±0·19	0·69±0·19	0·62±0·24	0·48±0·29
Adiponectin (µg/l)	4902±1154*	4612±944*	5588±932	5628±954	6253±818
HOMA index	27·2±8·0*	23·8±10·1	24·8±10·3	18·4±15·0	19·0±8·5

HF-L: lard-based, high-fat diet; HF-O: olive oil-based, high-fat diet; HF-C: coconut fat-based, high-fat diet; HF-F: fish oil-based, high-fat diet; SC: standard rodent chow. n=6 animals in all groups. *$P<0·05$ when compared to SC; #$P<0·05$ when compared to HF-F; ¶$P<0·05$ when compared to HF-C.

414–435 kJ per day (95% CI). Consequently, total food intake was 27%, 31% and 19% higher in HF-L, HF-O and HF-C respectively, when compared with SC ($P\leq0·05$). Significant differences between these three high-fat-diet types were not detected. Animals on fish oil-based diet (HF-F), however, showed high energy intake only during the first 4 weeks; this dropped to 335–419 kJ per day during the remaining feeding period. The resulting overall energy intake was only 5% higher than that in SC rats and significantly less than in HF-L (–17%, $P<0·05$) and HF-O (–19%, $P<0·05$) animals.

Weight gain

The weight gain per week is shown in Fig. 1B for all diet groups; the resulting final weight is given in Table 1. SC rats gained 49 ± 11 g body weight per week in the first 2 weeks. After this, the weekly weight gain fell linearly to less than 10 g at week 12. HF-L animals showed the highest weight gain of all groups with weekly increments of 67 ± 11 g in the early diet phase. As in the SC controls, weekly weight gain dropped to less than 10 g in the final diet week. The resulting final weight was 20 ± 11% higher than that of SC controls. The corresponding weight gain increments for HF-O and HF-C rats were slightly, but not significantly, lower than in HF-L. Final weights in these groups were 14 ± 11% and 9 ± 8% higher than SC ($P\leq0·05$); HF-L animals were 10 ± 8% heavier than HF-C rats ($P\leq0·05$). In HF-F rats, the weekly weight gain tended to be lower in HF-F than in SC, resulting in a significant 15 ± 11% decrease in final body weight.

Insulin tolerance tests

The degree of whole-body insulin resistance was assayed in the different diet groups by performing insulin tolerance tests (Fig. 2). Glucose levels in SC rats dropped from 5·0 ± 0·3 mmol/l to minimally 3·7 ± 0·8 mmol/l at 30 min after intraperitoneal insulin injection. The insulin-induced glucose disposal (GD) estimated from the area under the glucose concentration curve was

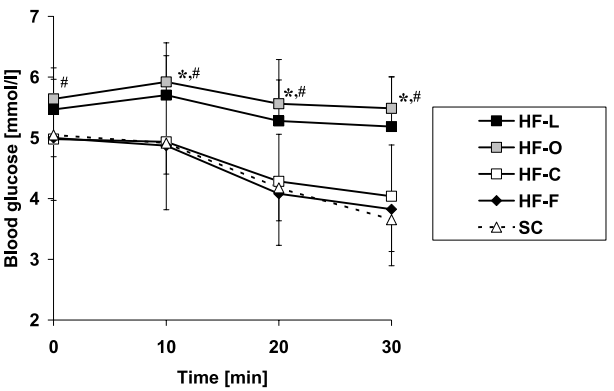

Figure 2 Insulin tolerance tests in high-fat-fed rats. After establishment of baseline blood glucose levels, rats were challenged intraperitoneally with 0·15 mU/kg insulin. Blood glucose levels were monitored for 30 min. Given are the means±S.D. of 12 rats per diet group. Black boxes: lard-based, high-fat diet (HF-L); gray boxes: olive oil-based, high-fat diet (HF-O); white boxes: coconut fat-based, high-fat diet (HF-C); black rhomboids: fish oil-based, high-fat diet (HF-F); white triangles: standard rodent chow (SC). *$P<0·05$ when comparing HF-L to SC; #$P<0·05$ when comparing HF-O to SC.

12 ± 6%. Mean glucose levels dropped less than 5% in HF-L and HF-O rats, leading to GD values of 1 ± 6% in both groups ($P \leq 0{\cdot}05$ compared with SC). The HF-C and HF-F animals showed a normal decrease of glucose levels in response to insulin with GD values of 8 ± 9% and 11 ± 5% respectively (P=n.s. compared with SC; $P \leq 0{\cdot}05$ compared with HF-L and HF-O).

Liver weight, histology and triglyceride content

The liver weight was 15–20% higher in the high-fat-fed rats ($P<0{\cdot}05$; Table 1). After normalization for total body weight, however, HF-L, HF-O and HF-C rats no longer differed from SC controls, whereas HF-F rats had about 35% larger livers than the other high-fat and the SC animals ($P \leq 0{\cdot}05$; Table 1). The histologic examination (hematoxylin–eosin staining) showed mainly microvesicular fat depositions in the HF-L, HF-O and HF-C livers (Fig. 3). No signs of inflammation or fibrosis were detected any group. As a measure of hepatic steatosis, the liver triglyceride content was markedly elevated in HF-L, HF-O and HF-C when compared with SC (3·9 ± 1·4-fold, 3·7 ± 0·9-fold, and 2·9 ± 0·9-fold respectively ($P \leq 0{\cdot}05$; Table 1)), while HF-F rats did not differ significantly from the controls.

Plasma characteristics

The plasma characteristics are given in detail in Table 1. Fasting glucose was moderately elevated in HF-O rats (12 ± 6%; $P \leq 0{\cdot}05$); the slight increase observed in HF-L rats was not statistically significant. HF-C and HF-F glucose levels did not differ from SC. Plasma triglycerides (TG) were highest in HF-C with a 2·3 ± 1·2-fold elevation over SC ($P \leq 0{\cdot}05$). TG were elevated about twofold in HF-L and HF-O, but this did not reach statistical significance; HF-F rats showed normal TG levels. Free fatty acids (FFA) were increased in HF-L, HF-O and HF-C, but this was statistically significant only in the HF-O animals (1·4 ± 0·3-fold increase over SC; $P \leq 0{\cdot}05$). Again, HF-F did not differ from SC controls. Creatinine as a marker of renal function and the plasma transaminases as markers of liver disease were not elevated in any of the high-fat-diet groups.

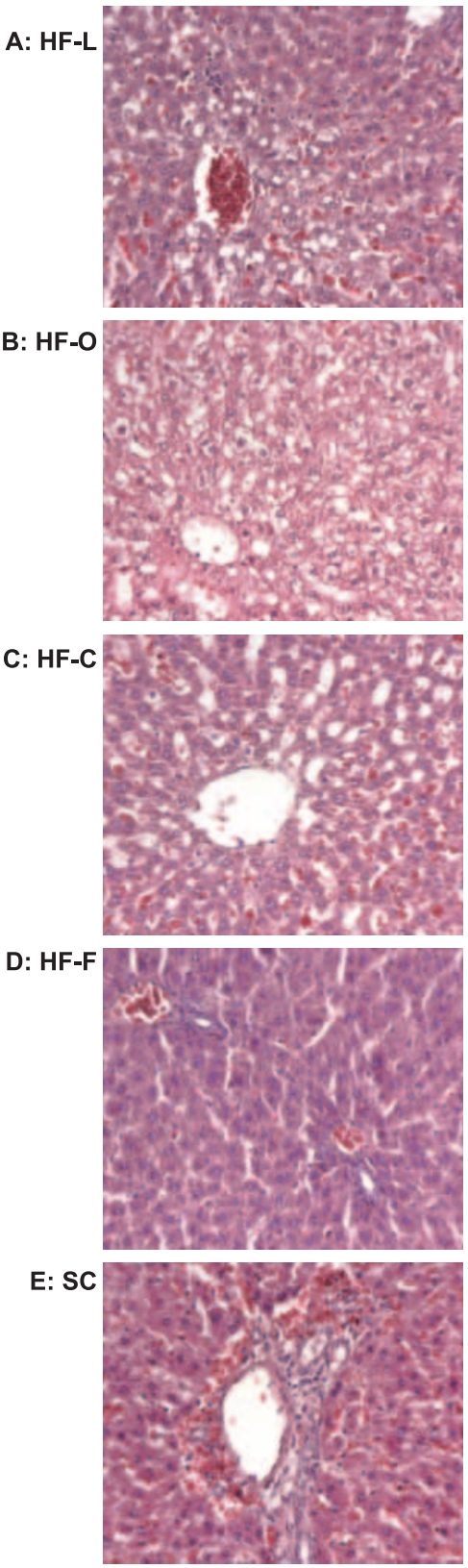

Figure 3 Liver histologies of high-fat-fed rats. Tissue samples were removed from the right ventral liver lobe directly postmortem and fixed in 10% neutral buffered formaldehyde. Representative HE stains (magnification 100) prepared according to standard procedures are shown from Wistar rats fed the lard-based, high-fat diet (HF-L) (A), olive oil-based, high-fat diet (HF-O) (B), coconut fat-based, high-fat diet (HF-C) (C), fish oil-based, high-fat diet (HF-F) (D), or standard rodent chow (SC) (E).

Table 2 Free fatty acid profiles after 12 weeks of dietary intervention. The values represent the absolute concentrations in nmol/l; given are the means±S.D. of six independent experiments

	HF-L	HF-O	HF-C	HF-F	SC
SFA					
Lauric acid	10±3¶,§	7±1$^{*,#,¶}$	127±42$^{*,#}$	8±1*	14±5
Myristic acid	18±12¶	11±4$^{#,¶}$	86±32$^{*,#}$	23±7	16±7
Palmitic acid	181±55$^{#}$	176±38$^{#}$	172±56	118±32	151±46
Stearic acid	52±14$^{*,#,¶}$	43±14$^{#}$	37±6$^{#}$	29±5	31±8
Sum	**260±82¶**	**236±54¶**	**422±136$^{*,#}$**	**178±43**	**212±55**
MUFA					
Palmitoleic acid	32±13$^{#}$	27±5$^{#,¶}$	49±19*	58±16*	25±16
Oleic acid	191±68*,§	316±71$^{*,#,¶}$	139±43*	157±53*	80±36
Sum	**223±80*,§**	**343±74$^{*,#,¶}$**	**189±61***	**215±68***	**105±52**
PUFA					
Linoleic acid	66±20$^{*,#,¶}$	49±14$^{*,#,¶}$	33±7$^{*,#}$	20±5*	133±43
Linolenic acid	4±1$^{*,#,¶,§}$	2±1$^{*,#,¶}$	1±1*	1±0*	12±5
Arachidonic acid	45±10$^{#}$	51±17$^{#}$	40±5$^{#}$	17±2*	36±5
Eicosapentaenoic acid	0±0$^{*,#}$	1±1$^{#}$	0±0$^{*,#}$	6±3*	0±0
Docosahexaenoic acid	6±5$^{#,¶}$	8±3$^{#,¶}$	0±0$^{*,#}$	16±4*	5±4
Sum	**121±26$^{*,#,¶}$**	**112±33$^{*,#,¶}$**	**74±8***	**60±5***	**187±53**
Sum ω-6	**111±25$^{*,#,¶}$**	**100±28$^{*,#}$**	**73±8$^{*,#}$**	**36±5***	**169±47**
Sum ω-3	**10±4$^{*,#,¶}$**	**11±5$^{*,#,¶}$**	**1±1$^{*,#}$**	**23±6**	**18±7**

HF-L: lard-based, high-fat diet; HF-O: olive oil-based, high-fat diet; HF-C: coconut fat-based, high-fat diet; HF-F: fish oil-based, high-fat diet; SC: standard rodent chow; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n=6 animals in all groups. $^{*}P<0{\cdot}05$ when compared to SC; $^{\#}P<0{\cdot}05$ when compared to HF-F; $^{¶}P<0{\cdot}05$ when compared to HF-C; $^{§}P<0{\cdot}05$ when compared to HF-O.

In endocrine parameters, we found a $35\pm39\%$ increase of plasma insulin and a $67\pm18\%$ increase of glucagon ($P\leq0{\cdot}05$) in the HF-L diet group. Insulin and glucagon levels were elevated in HF-O and HF-C rats, too, but statistical significance could not be established because of the relatively high interindividual variability. Plasma adiponectin was decreased by $22\pm18\%$ and $24\pm15\%$ in the HF-L and HF-O groups ($P\leq0{\cdot}05$); HF-C and HF-F rats did not show clear reductions in adiponectin levels. The HOMA index, which reflects whole body insulin resistance, was increased in HF-L animals ($1{\cdot}4\pm0{\cdot}4$-fold; $P\leq0{\cdot}05$). HF-C and HF-O both showed about 1·3-fold elevated HOMA values, but this was not statistically significant.

Plasma fatty acid profile

The absolute concentrations of the major free fatty acids are shown in Table 2. HF-L rats had tendentially higher levels of palmitic and stearic acid, and significantly higher oleic acid levels than SC controls ($2{\cdot}4\pm0{\cdot}9$-fold; $P\leq0{\cdot}05$). Linoleic and linolenic acid levels were decreased by $50\pm15\%$ and $32\pm11\%$ respectively ($P\leq0{\cdot}05$). HF-O rats showed the highest levels of oleic acid (more than threefold over SC); the other fatty acid concentrations were comparable to HF-L. In HF-C animals, total SFA levels were the highest among the examined diets (about double those of SC), and relatively short long-chain SFA predominated: lauric acid (C_{12}) levels were 8–16-fold higher than in the other groups, and myristic acid (C_{14}) levels 4–7-fold higher ($P\leq0{\cdot}05$). Interestingly, MUFA levels were also higher than in SC rats ($1{\cdot}8\pm0{\cdot}6$-fold; $P\leq0{\cdot}05$). In HF-F rats, SFA levels were generally comparable to SC, and MUFA levels had approximately doubled. Major increases were observed in the levels of the maritime ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acid). All other diet groups, including the SC controls, showed about 70–80% lower concentrations of this fatty acid type. To analyze associations between single fatty acids and fatty acid classes with metabolic parameters, we correlated the absolute levels of the examined fatty acids with weight, fasting glucose and insulin, and GD (Table 3). We found a moderate to high positive correlation between palmitic, stearic and arachidonic acid and the animals' weight and GD, and a high negative correlation between weight and the maritime ω-3 fatty acids. Arachidonic acid also was positively correlated with fasting glucose and insulin levels, whereas these parameters were negatively associated with palmitoleic and eicosapentaenoic acid. When we performed the correlations while controlling for weight, only the positive association between arachidonic acid and fasting glucose and insulin levels remained significant (r=0·48 and 0·37 respectively; $P\leq0{\cdot}05$).

Table 3 Correlations between specific free fatty acid plasma levels and metabolic parameters. Given are Pearson's correlation coefficients

	Weight	Fasting glucose	Plasma insulin	Glucose disposal
SFA				
Lauric acid	0·15	−0·09	0·16	−0·11
Myristic acid	0·09	−0·17	0·03	−0·16
Palmitic acid	0·44*	0·29	0·06	0·28
Stearic acid	0·56*	0·33	0·29	0·4*
Sum	**0·34***	**0·08**	**0·14**	**0·07**
MUFA				
Palmitoleic acid	−0·34	−0·41*	−0·45*	−0·13
Oleic acid	0·29	0·34	0·01	0·43*
Sum	**0·21**	**0·25**	**−0·08**	**0·39***
PUFA				
Linoleic acid	0·09	0·17	0·03	−0·07
Linolenic acid	−0·02	0·08	−0·08	−0·12
Arachidonic acid	0·70*	0·65*	0·53*	0·48*
Eicosapentaenoic acid	−0·72*	−0·38*	−0·30	−0·33
Docosahexaenoic acid	−0·58*	−0·26	−0·23	−0·08
Sum	**0·17**	**0·29**	**0·13**	**0·04**
Sum ω-6	**0·29**	**0·34**	**0·18**	**0·08**
Sum ω-3	**−0·64***	**−0·26**	**−0·30**	**−0·22**

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. $n=6$ in all diet groups. *$P<0·05$.

Hepatic expression of genes involved in glucose and lipid metabolism

Liver samples from two animals in each diet group were subjected to mRNA expression analysis on the Affymetrix Rat 230 V.2 GeneChip. Overall, 190, 256, 231 and 269 genes were downregulated, and 101, 108, 93 and 123 genes were upregulated in HF-L, HF-O, HF-C and HF-F respectively. Detailed data concerning genes related to lipid and glucose metabolism are shown in Tables 4 and 5. Most significant changes induced by the high-fat diets were seen in the lipid synthesis and metabolism, and the fatty acid oxidation subgroups. Comparing the different high-fat diets with each other, we found key genes of lipid synthesis, such as fatty acid synthase or stearoyl desaturase, to be upregulated in HF-L, HF-O and HF-C, the effect being quantitatively strongest in HF-O. In HF-F animals, some liposynthetic genes, such as stearoyl desaturase, were also upregulated, but, in general, there was no significant change to SC. In contrast, major enzymes of fatty acid oxidation, such as carnitine palmitoyl transferase or enoyl-coA hydratase, were downregulated in HF-L, HF-O and HF-C, but not changed or upregulated in HF-F. The major transcriptional regulator of hepatic fatty acid synthesis, SREBP1c (sterol response element-binding protein 1c), was consistently upregulated in all diet groups, whereas PPARα (peroxisome proliferator activated receptor α), a key regulator of fatty acid oxidation, was upregulated only in HF-F. To confirm the relevance of SREBP1c and PPARα for the expression changes observed, we quantified the number of diet-regulated SREBP1c- and PPARα-dependent genes, as well as the respective numbers of genes regulated by the transcription factors HNF-4α (hepatic nuclear factor 4α) and LXR (liver X receptor), which are also implicated in the regulation of hepatic lipid metabolism (Fig. 4). The greatest upregulation of SREBP1c-dependent genes was observed in HF-O rats (9/10 examined genes), followed by HF-L and HF-C (both 8/10 examined genes). In HF-F, only 3/10 SREBP1c-dependent genes were upregulated. In contrast, 8/15 PPAR-dependent genes included in the analysis were upregulated in HF-F. The other HF diets induced only minor expression changes in the respective genes: HF-L rats showed an upregulation of four PPARα-dependent genes, and HF-C and HF-O of only one. HNF-4α- and LXR-dependent genes were not differentially expressed in the different diet types.

Validation of the GeneChip analysis

Given the multitude of differentially regulated genes, it was not possible to verify all changes by a second independent method. We therefore spot-checked the data by performing quantitative LightCycler RT–PCR on the genes for the most important liposynthetic regulators, SREBP1c and PPARα, and a subset of genes regulated by these two transcription factors,

Table 4 Affymetrix gene expression profile analysis – selected genes from lipid synthesis and metabolism

			HF-L		HF-O		HF-C		HF-F	
	Regulator	UniGene-ID	Change call	(fold change)	Change call	(fold change)	Change call	(fold change)	Change call	(fold change)
Gene										
Lipid synthesis and metabolism										
Malic enzyme	SREBP1	Rn.64900	I	3·3±0·1	I	7·4±1·6	I	3·1±0·6	I	5·5±1·1
Fatty acid synthase	SREBP1	Rn.9486	I	6·1±1·9	I	22·5±3·4	I	10·4±5·3	NC	
Diacylglycerol acyltransferase		Rn.252	NC		NC		NC		NC	
Pyruvate kinase		Rn.48821	I	2·4±0·5	I	4·0±0·9	I	2·5±0·5	NC	
Glycerol-3-phosphate acyltransferase	SREBP1	Rn.44456	I	2·1±0·2	I	2·8±0·7	NC		NC	
Long-chain acetyl CoA synthetase	SREBP1	Rn.6215	NC		NC		NC		NC	
Acetyl-coenzyme A carobxylase	SREBP1	Rn.122519	I	1·8±0·3	I	4·7±1·0	I	2·7±0·5	NC	
Lipoprotein lipase	LXR	Rn.3834	NC		NC		NC		NC	
Stearoyl CoA desaturase	SREBP1	Rn.1023	I	4·9±1·7	I	7·8±2·5	I	6·9±2·3	I	4·5±2·1
Lecithin-cholesterol acyltransferase		Rn.10481	NC		NC		NC		NC	
Hepatic lipase		Rn.1195	NC		NC		NC		NC	
ATP citrate lyase	SREBP1	Rn.29771	I	2·1±0·4	I	4·9±1·8	I	1·9±0·7	NC	
Carboxylesterase 1 (ES-3)		Rn.82692	D	0·7±0·1	D	0·6±0·0	D	1·0±0·1	NC	
Carboxylesterase 3		Rn.34885	D	0·4±0·1	D	0·2±0·0	D	0·5±0·1	NC	
AMP-activated protein kinase, beta-1 subunit		Rn.3619	NC		NC		NC		NC	
AMP-activated protein kinase, beta-2 subunit		Rn.48744	NC		NC		NC		NC	
AMP-activated protein kinase, alpha-1 subunit		Rn.87789	NC		NC		NC		NC	
Fatty acid elongase 1	SREBP1	Rn.4243	I	1·9±0·1	I	1·9±0·2	I	2·0±0·3	I	1·7±0·1
Fatty acid elongase 2	SREBP1	Rn.46942	NC		NC		I	2·9±0·6	NC	
Fatty acid desaturase 2		Rn.32872	I	3·0±0·4	I	3·3±0·1	I	2·7±0·3	I	3·1±0·1
Fatty acid oxidation										
Carnitine O-octanoyltransferase	PPARα	Rn.4896	NC		NC		NC		I	1·5±0·1
Peroxisomal membrane protein Pmp26p	PPARα	Rn.14519	I	2·3±0·5	NC		NC		I	3·3±0·8
Carnitine palmitoyl transferase	PPARα	Rn.11389	D	0·7±0·0	D	0·6±0·0	D	0·8±0·0	NC	
Mitochondrial multienzyme complex (B-subunit)	PPARα	Rn.11253	NC		NC		NC		I	1·5±0·1
Enoyl coenzyme A hydratase	PPARα	Rn.6148	NC		D	0·6±0·2	NC		I	2·2±0·4
Acetyl-CoA acyltransferase		Rn.8913	NC		NC		NC		I	2·4±0·3
Acyl-CoA oxidase	PPARα	Rn.31796	NC		NC		NC		I	1·5±0·0
Very long-chain Acyl-CoA dehydrogenase	PPARα	Rn.33319	NC		NC		NC		I	1·2±0·1
Medium-chain Acyl-CoA dehydrogenase	PPARα	Rn.6302	NC		D	0·8±0·0	NC		NC	
Acyl-coenzyme A dehydrogenase, short-chain	PPARα	Rn.44423	D	0·7±0·1	D	0·6±0·0	D	0·7±0·0	D	0·8±0·0
Acetyl-coenzyme A acyltransferase 2		Rn.3786	NC		NC		NC		NC	
Acetyl-coenzyme A dehydrogenase, long-chain		Rn.174	I	1·5±0·1	NC		I	1·4±0·0	I	1·6±0·1
Fatty acid coenzyme A ligase, long-chain 5	PPARα	Rn.105862	I	2·4±0·1	I	3·1±0·3	I	2·2±0·4	I	2·1±0·1
Peroxisomal multifunctional enzyme type II		Rn.2082	NC		NC		NC		NC	
Dodecenoyl-coenzyme A delta isomerase	PPARα	Rn.80835	NC		NC		NC		I	2·2±0·5
Uncoupling protein 2		Rn.3333	NC		NC		NC		NC	
Acyl-CoA thioesterase 1	PPARα	Rn.11326	NC		NC		NC		I	4·8±1·0
Fatty acid transport										
CD36 (fatty acid translocase)	PPARα	Rn.3790	I	3·0±0·7	I	4·2±0·5	NC		I	7·4±0·4
ATP-binding cassette ABCD3		Rn.7024	NC		NC		NC		NC	
Apolipoproteins										
Apolipoprotein A-I		Rn.10308	NC		I	1·9±0·3	I	1·7±0·2	I	1·7±0·3
Apolipoprotein C-III	HNF-4α	Rn.36813	NC		NC		NC		NC	
Apolipoprotein C-I		Rn.8887	NC		NC		NC		NC	
Apolipoprotein A-II	HNF-4α	Rn.89304	NC		NC		NC		NC	
Apolipoprotein E	LXR	Rn.32351	NC		NC		NC		NC	
Apolipoprotein B		Rn.33815	NC		NC		NC		NC	
Apolipoprotein AIV	HNF4α	Rn.15739	NC		NC		NC		D	0·2±0·0
Apolipoprotein M		Rn.262	NC		NC		NC		NC	
Transcriptional regulation										
SREBP1		L16995	I	4·5±0·6	I	6·1±0·7	I	7·2±0·6	I	3·4±0·8
Farnesoid X activated receptor		Rn.42943	NC		NC		NC		NC	
NFY-C		Rn.1457	NC		NC		NC		NC	
HNF-4α		Rn.44442	NC		D	0·9±0·1	D	0·9±0·1	NC	

Table 4 *Continued*

Gene	Regulator	UniGene-ID	HF-L Change call (fold change)	HF-O Change call (fold change)	HF-C Change call (fold change)	HF-F Change call (fold change)
Transcriptional regulation *continued*						
USF1		Rn.37514	NC	NC	NC	NC
NFY-B		Rn.1131	NC	NC	NC	NC
NrOb2		Rn.10712	NC	NC	NC	D 0·3±0·0
PPARγ		Rn.23443	NC	NC	NC	NC
Cbp/p300-interacting transactivator 2		Rn.31765	NC	NC	I 1·9±0·2	NC

Change call column shows expression changes in the different diet groups when compared to SC (NC=no change, I=increase, D=decreased); the actual fold change (±S.E.M.) is shown for genes with significantly increased or decreased mRNA levels. The Regulator column shows whether the gene was counted as predominantly regulated by SREBP1, PPARα, HNF-4α or LXR in the subsequent analysis. It does not give a complete listing of all known transcriptional regulators.

acetyl-CoA-dehydrogenase (very long chain), glycogen synthase, glucokinase, fatty acid synthase and enoyl-CoA-hydratase, in the livers of six animals. The results validated the array analysis. Hepatic SREBP1c mRNA content was elevated 1·5- (HF-F), 1·7- (HF-L), 2·2- (HF-O) and 3·0-fold (HF-C) when compared with SC ($P \leq 0{\cdot}05$), whereas PPARα mRNA abundance was increased moderately and without statistical significance only in HF-F rats (1·5-fold; $P=0{\cdot}13$). Glucokinase and fatty acid synthase gene expression was upregulated, and glycogen synthase expression was significantly down-regulated in HF-L, HF-C and HF-O, whereas enoyl-CoA hydratase and acetyl-CoA-dehydrogenase expression was upregulated in HF-F (data not shown).

Insulin secretion *ex vivo*

Basal insulin secretion at ('normoglycemic') 5·6 mM glucose was reduced by 30–60% when we compared isolated islets from high-fat-fed animals with SC controls. This inhibitory effect was observed for each high-fat regimen at a significance level of $P \leq 0{\cdot}05$; significant differences between the single high-fat diets were not found (Fig. 5).

Discussion

Fat-enriched diets have been used for decades to model obesity, dyslipidemia and insulin intolerance in rodents. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this also may extend to the cardiovascular complications (Aguila & Mandarim-de-Lacerda 2003, Woods *et al.* 2003). Basically, all laboratory rodent species are prone to develop metabolic perturbations under such dietary regimens (Sullivan *et al.* 1993, Tschop & Heiman 2001). High-fat-diet susceptibility, that is, the extent of the metabolic disorder induced by the respective diet, depends more on the specific rodent strain and the dietary regimen employed than on the species itself. For example, C57BL/6J mice develop obesity and insulin resistance similar to Wistar rats, while 129S6 (Almind & Kahn 2004) or A/J mice (Surwit *et al.* 1988) do not. To our knowledge, the precise mechanism of this high-fat resistance observed in some rat and mouse strains is not understood yet.

Many mouse studies have examined genetic modifications in the context of a high-fat diet, while the majority of 'purely' metabolic high-fat-diet studies in wild-type animals have been performed in rats (Medline search, January 2006). In these latter studies, the severity of the metabolic perturbation (weight gain, glucose, insulin and adipokine levels, insulin resistance, etc.) described by previous authors agrees well with our results (see below), but the large range of the individual results also reflects the heterogeneity of the respective experimental designs.

Therefore, given the small number of previous, comprehensive, high-fat-diet comparisons, the first aim of this study was to characterize systematically the effects of high-fat diets differing only by their main fat component on the induction of a metabolic syndrome in Wistar rats. From the animals' phenotype and the parameters of glucose metabolism and insulin action, it appears that both the HF-L and the HF-O diets led to the most pronounced manifestations of obesity and insulin resistance. The animals in these groups gained more weight, had higher plasma glucose levels and showed less efficient, insulin-induced glucose disposal than animals fed coconut fat, fish oil or standard rat chow. This result is not unexpected as far as the lard-based diet is concerned, as it corroborates a large number of earlier studies of high-fat diets based on this fat type (Buettner *et al.* 2000, 2004, Yaspelkis *et al.* 2001, Gustafson *et al.* 2002, Briaud *et al.* 2002). The decrease in adiponectin levels and the increase in plasma glucagon

Table 5 Affymetrix genechip expression profile analysis – selected genes from cholesterol, glucose metabolism and TCA cycle

			HF-L		HF-O		HF-C		HF-F	
	Regulator	**UniGene-ID**	Change call	(fold change)	Change call	(fold change)	Change call	(fold change)	Change call	(fold change)
Gene										
Cholesterol related										
Cholesterol-7-hydroxylase (Cyp7a1)	LXR, HNF-4α	Rn.10737	NC		NC		NC		NC	
Scavenger receptor class B, member 1		Rn.3142	NC		NC		NC		NC	
Lecithin:cholesterol acyltransferase (LCAT)		Rn.10481	NC		NC		NC		NC	
HMG-CoA reductase		Rn.10469	NC		NC		NC		NC	
HMG-CoA synthase		Rn.5106	NC		NC		NC		NC	
ATP-binding cassette subfamily ABCB11	PPARα	Rn.14539	D	0·7±0·0	D	0·8±0·0	D	0·7±0·0	D	0·6±0·0
ATP-binding cassette subfamily ABCC9		Rn.10528	NC		NC		NC		NC	
ATP-binding cassette subfamily ABCC2		Rn.10265	D	0·5±0·0	D	0·6±0·0	D	0·6±0·1	D	0·7±0·1
Low-density lipoprotein receptor		Rn.10483	NC		NC		NC		NC	
Cyp7b1		Rn.53969	NC		NC		NC		NC	
Insulin-related genes										
Prolactin receptor		Rn.9757	I	17·4±7·0	I	18·2±7·0	NC		I	12·5±3·7
Insulin receptor-related receptor		Rn.44446	NC		NC		NC		NC	
IRS-1		Rn.10476	NC		NC		D	0·6±0·0	NC	
IRS-3		Rn.9791	I	5·7±1·1	I	6·4±1·5	NC		NC	
IGF-1		Rn.6282	NC		NC		NC		NC	
PI-3 kinase		Rn.30010	NC		D	0·7±0·1	NC		D	0·4±0·0
PI-3 kinase reg. Subunit, pp 1		Rn.10599	NC		NC		NC		NC	
Protein phosphatase 1		Rn.39034	NC		NC		NC		NC	
S6 kinase		Rn.4042	NC		NC		NC		NC	
Protein kinase B		Rn.11422	NC		NC		NC		NC	
3-phosphoinositide dependent protein kinase-1		Rn.10905	NC		NC		NC		NC	
Glycogen synthase kinase 3 beta		Rn.10426	NC		NC		NC		NC	
MAPK-1		Rn.34914	NC		NC		NC		NC	
MAPK-3		Rn.2592	NC		NC		NC		NC	
Insulin-like growth factor binding protein 1		Rn.34026	NC		NC		NC		NC	
Glycloysis – gluconeogenesis										
Glucokinase	SREBP1	Rn.10447	I	13·3±1·6	I	8·4±2·9	I	21·1±5·7	NC	
Phosphofructokinase, liver, B-type		Rn.4212	NC		NC		NC		NC	
Glutamate oxaloacetate transaminase 2		Rn.98650	NC		NC		NC		NC	
Fructose-1,6- biphosphatase 1		Rn.33703	NC		NC		NC		NC	
Glutamate oxaloacetate transaminase 1		Rn.5819	D	0·3±0·0	D	0·3±0·0	D	0·3±0·0	D	0·4±0·0
Pyruvate kinase	HNF-4α	Rn.48821	I	2·4±0·5	I	4·0±0·9	I	2·5±0·5	NC	
Glucose-6-phosphatase, catalytic		Rn.10992	NC		NC		NC		NC	
Phosphoenolpyruvate carboxykinase	HNF-4α	Rn.104376	NC		NC		NC		NC	
Glycogen metabolism										
Glutathione peroxidase 2		Rn.3503	NC		D	0·5±0·2	NC		NC	
Phosphorylase kinase, gamma 2 (testis)		Rn.11153	NC		NC		NC		NC	
Glycogenin		Rn.3661	I	1·5±0·2	I	1·5±0·0	I	1·4±0·1	I	1·5±0·1
Protein phosphatase 2, regulatory subunit		Rn.81155	NC		NC		NC		NC	
Muscle glycogen phosphorylase		Rn.11238	NC		NC		NC		NC	
Phosphorylase kinase gamma 1		Rn.10399	NC		NC		NC		NC	
Protein phosphatase 2, regulatory subunit B		Rn.81155	NC		NC		NC		NC	
Protein phosphatase 2, catalytic subunit		Rn.1271	NC		NC		NC		NC	
Phosphorylase B kinase alpha subunit		Rn.48743	NC		NC		NC		NC	
Glycogen synthase 2		Rn.2906	D	0·5±0·0	D	0·7±0·0	D	0·7±0·0	NC	
TCA cycle										
Mitochondrial aconitase (nuclear aco2 gene)		Rn.43737	D	0·8±0·0	NC		NC		NC	
Fumarate hydratase 1		Rn.29782	D	0·7±0·0	D	0·8±0·0	NC		NC	
Pyruvate dehydrogenate kinase 4		Rn.30070	NC		NC		NC		NC	
Pyruvate dehydrogenase kinase 2		Rn.88597	NC		NC		NC		NC	

Change call shows expression changes in the different diet groups when compared to SC (NC=no change, I=increase, D=decreased); the actual fold change (±S.E.M.) is shown for genes with significantly increased or decreased mRNA levels. The Regulator column shows whether the gene was counted as predominantly regulated by SREBP1, PPARα, HNF-4α or LXR in the subsequent analysis. It does not give a complete listing of all known transcriptional regulators.

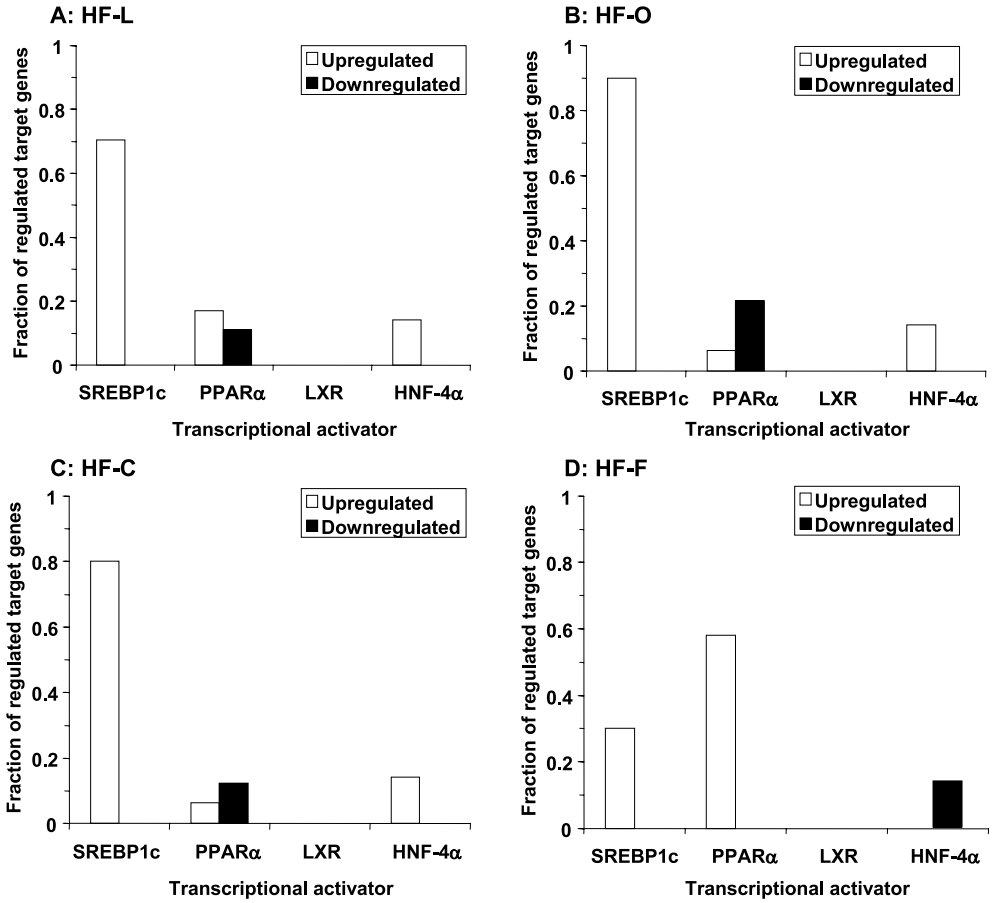

Figure 4 Hepatic gene expression analysis: relative fractions of up- and downregulated SREBP1c-, PPARα-, HNF-4α- and LXR-dependent genes. The relative hepatic mRNA abundance of 10 SREBP1c-regulated, 15 PPARα-regulated, three LXR-regulated and seven HNF-4α-regulated genes (see Tables 4 and 5 for the respective gene names) was measured by Affymetrix GeneChip analysis. The figures show the fraction of up- or downregulated genes in Wistar rats fed the lard-based, high-fat diet (HF-L) (A), olive oil-based, high-fat diet (HF-O) (B), coconut fat-based, high-fat diet (HF-C) (C) or fish oil-based, high-fat diet (HF-F) (D).

have not been described before for this particular diet, and both phenomena could contribute to the development of insulin resistance (Weyer *et al.* 2001, Pajvani & Scherer 2003). As described previously, hepatic steatosis was induced by the HF-L diet (Buettner *et al.* 2004). Histologic examination of the liver did not reveal signs of inflammation or distinct fibrotic changes. As all of these phenomena have also been described in human obesity, they confirm the usefulness of lard-based, high-fat models to induce a metabolic syndrome-like phenotype.

Olive oil and coconut fat have been used much less frequently in rodent high-fat diets. From a clinical point of view, one might expect an olive oil-based diet (containing mainly MUFA) to induce less negative metabolic effects than lard (containing both SFA and MUFA) or coconut fat (containing mainly SFA), as current nutrition recommendations include reduction of saturated fat intake, whereas plant oils containing MUFA are to be favored. This notion is derived mainly from cohort studies demonstrating the positive impact of the so-called Mediterranean diet on cardiovascular morbidity and mortality (Kris-Etherton 1999). Consistent with our results, previous animal studies have found insulin resistance and elevations of plasma lipids in rats fed high-fat diets based on olive oil (Del Moral *et al.* 1997, Storlien *et al.* 1991, Tsunoda *et al.* 1998). The evidence of specific positive effects of monounsaturated fats on glucose and lipid metabolism is not very conclusive. Early interventions with high-fat, olive oil diets in man have shown improvements of glycemic control and insulin resistance (Parillo *et al.* 1992, Campbell *et al.* 1994), but this may be attributed to the generally positive, short-term effects of high-fat/low-carbohydrate diets on body weight and

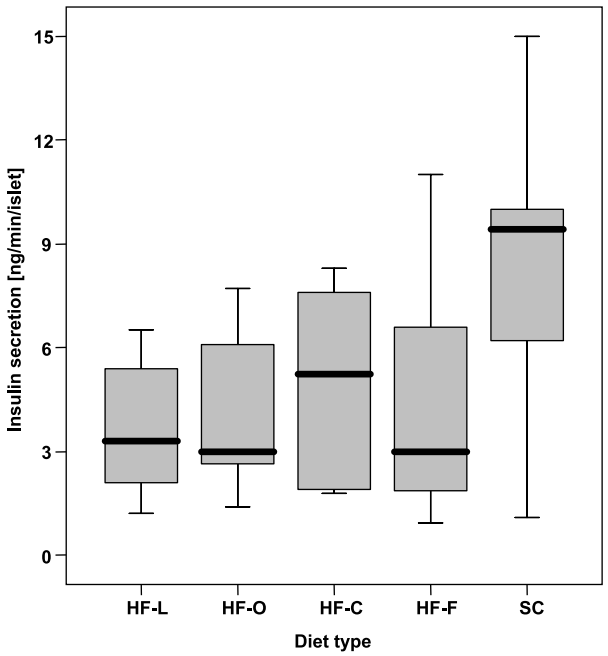

Figure 5 Insulin secretion from islets isolated from high-fat-fed rats. Pancreatic islets were isolated from high-fat-fed rats and incubated in Krebs-Ringer bicarbonate solution (5·6 mM glucose), as described in the text. The insulin secretion per minute and islet (calculated from the supernatant insulin levels after 75 and 90 min) is given as box-whisker plot for the different diet groups. HF-L: lard-based, high-fat diet; HF-O: olive oil-based, high-fat diet; HF-C: coconut fat-based, high-fat diet; HF-F: fish oil-based, high-fat diet; SC: standard rodent chow. *$P \leq 0{\cdot}05$ when compared with SC.

glucose metabolism, as recently described (Samaha *et al.* 2003). Adherence to a classical Mediterranean diet has recently been associated with lower insulin resistance parameters (Esposito *et al.* 2004). It must be kept in mind, however, that this diet, rich in whole grains, fruits and vegetables, typically has a low-fat composition. Consequently, the usefulness of a 'Mediterranean high-fat diet' has been questioned (Ferro-Luzzi *et al.* 2002). In summary, the metabolic impact of high oleic acid consumption is not defined unequivocally in man, whereas it clearly leads to obesity and insulin resistance in rats. For modeling the metabolic syndrome by a high-fat diet, olive oil does not seem to have a distinct advantage over lard.

It is generally believed that high intake of saturated fat is a major cause of the development of the metabolic syndrome. However, in our study, the measures of obesity and insulin resistance were not as clearly elevated in the coconut fat-fed animals as in the lard- or olive oil-fed rats. This was accompanied by profoundly elevated plasma triglyceride levels and mild hepatic steatosis. While increased liver fat deposition and elevation of circulating lipids have been described previously with this dietary fat (Feoli *et al.* 2003, Wood 2004), these studies have not conclusively shown that high intake of the relatively short (C_{12}, C_{14}), long-chain, saturated fatty acids predominant in coconut fat – that is, lauric and myristic acids – damages glycemic control. At least one former study has found lowered plasma glucose levels in such animals (Zulet *et al.* 1999). The notion that diets high in saturated fat induce the most pronounced insulin resistance in rats goes back to a study by Storlien *et al.* (1991). Here, the diet type referred to as *saturated* consisted of a tallow/safflower oil mixture, and the relative content of saturated fat in this diet was only 19%. To our knowledge, no other study has directly measured parameters of insulin action *in vivo* in coconut fat-fed rats. In man, myristic acid levels correlate positively with insulin levels, but not with the HOMA index (Lovejoy *et al.* 2001); an experimental elevation of coconut fat intake does not lead to insulin resistance (Schwab *et al.* 1995); and chronically high coconut fat consumption, as in traditional Polynesian diets, is not associated with elevated diabetes prevalence (Taylor *et al.* 1983). Thus, it seems conceivable that high intake of C_{12} and C_{14} saturated fatty acids may not be necessarily deleterious to glucose metabolism. In view of coconut fat-induced hypertriglyceridemia, however, it does not seem appropriate to advocate unconditionally this dietary fat source for nutritional interventions.

The positive effects of fish oil-based diets on lipid and glucose metabolism, such as lower plasma triglyceride, glucose and insulin levels, and more effective glucose disposal, have been described extensively before (Storlien *et al.* 2000, Delarue *et al.* 2004). In our study, we also clearly observed less weight gain in these animals than in the standard chow-fed controls, an effect that was not explained by a lower caloric intake. Whether this was due to reduced resorption of dietary calories in the HF-F rats or to elevation of energy expenditure cannot be decided from our data, but previous studies point to enhanced thermogenesis in fish oil-fed rats through increasing the expression of mitochondrial uncoupling proteins and increasing fatty acid oxidation by the less efficient peroxisomal pathway (Oudart *et al.* 1997, Baillie *et al.* 1999). Hepatomegaly induced by fish oil-feeding has been described consistently before (Otto *et al.* 1991, Yaqoob *et al.* 1995, Rabbani *et al.* 2001, Nakatani *et al.* 2003), but the extent of hepatic fat deposition in fish oil-fed rats remains controversial. Some studies report an elevated (Otto *et al.* 1991, Yaqoob *et al.* 1995) and others a lowered liver lipid content (Levy *et al.* 2004), a finding that might reflect either differences induced by the specific dietary source or rat strain-dependent variability. In our study, we were able to demonstrate distinct liver enlargement in HF-F rats without signs of major hepatic steatosis or elevation of transaminases. Given the normal hepatic histologic architecture, it can be speculated that fish oil might

induce liver cell hyperplasia, but, to our knowledge, no experimental data support this hypothesis to date.

Although not the direct focus of the present study, we made an interesting observation in comparing the hyperinsulinemia found in high-fat rats *in vivo* with the decrease in insulin secretion at 5·6 mM glucose found in corresponding isolated pancreatic islets. These *ex vivo* findings indicate that high-fat diets might compromise the pancreatic beta cell secretory function already at normoglycemic stages of the progression from normal glucose tolerance to insulin resistance and overt diabetes mellitus. It can be speculated that *in vivo* systemic insulinotropic factors (e.g. free fatty acids) may overcome the impairment of pancreatic insulin secretion in order to compensate for peripheral insulin resistance. However, this hypothesis certainly needs further investigation.

The plasma free fatty acid profiles in the different experimental groups mirrored the fatty acid composition of their respective diets, as has been described before (Yaqoob *et al.* 1995). In an overall analysis, we tried to correlate the plasma concentrations of single free fatty acids with different metabolic parameters. After correction for obesity, only a moderate association between arachidonic acid and fasting glucose and insulin levels remained significant. These data do not show specific deleterious or advantageous actions of selected saturated or monounsaturated fatty acids. The negative impact of arachidonic acid, however, might be explained by its proinflammatory actions. Some experimental data suggest NFκ-B activation by arachidonic acid in monocytes and hepatocytes (Camandola *et al.* 1996, Becuwe *et al.* 2003), which in turn plays an important role in the pathogenesis of local and systemic insulin resistance (Arkan *et al.* 2005, Cai *et al.* 2005).

Fatty acids might exert their intracellular effects through various mechanisms, including – but not limited to – changes in membrane composition, intracellular metabolite levels and eicosanoid production (Sampath & Ntambi 2005). They are implicated in the regulation of gene transcription (Pegorier *et al.* 2004), and the liver is a main site of fatty acid metabolism. Looking at the differences in plasma fatty acid composition induced by the different high-fat diets, we therefore examined by GeneChip analysis the extent to which the hepatic gene expression profile was influenced by the diet type. In terms of the mRNA abundance of 'metabolic' genes, lard, olive oil and coconut fat did not differ significantly from each other in many respects: HF-L, HF-O and HF-C all preferentially induced increases in hepatic liposynthetic gene expression; the expression of genes related to fat oxidation was partly up- and partly downregulated in these three diet groups; and the key enzymes of glycolysis, glucokinase and pyruvate kinase were concordantly upregulated. In summary, in these three diets, typical hepatic insulin actions, such as lipogenesis and glycolysis, were transcriptionally enforced, whereas HF-F-fed rats showed enhanced expression of genes related to lipid oxidation. Of the four main transcription factor families implicated in gene expression regulation by fatty acids (PPAR, SREBP, LXR and HNF-4α), only the mRNA abundance of SREBP1c, the main hepatic transcriptional regulator of fatty acid synthesis, was consistently increased in all high-fat-fed groups; PPARα mRNA was trendwise elevated in HF-F rats. Distinct expression changes were observed for SREBP1c- and PPARα-dependent gene clusters, whereas LXR- and HNF-4α regulated genes were not differentially expressed under high-fat diets. No clear expression change of SREBP2 and the dependent genes of cholesterol synthesis was observed in any diet type. This points to a less important role for the latter transcription factors in the dysregulation of hepatic fat and glucose metabolism seen in this model system.

Elevated gene expression of SREBP1c has been described before in other dietary and genetic models of fatty liver (Shimomura *et al.* 1999, Becker *et al.* 2004, Lin *et al.* 2005). In one recent study examining long-term, high-fat feeding, SREBP1c transcription and lipogenesis were not increased. Comparison with our data is not possible, however, as the dietary fat composition is not stated in that publication (Kim *et al.* 2004). SREBP1c expression is increased by insulin and LXR and downregulated by glucagon, leptin and PUFA (Shimano 2001, Cagen *et al.* 2005). Despite high oleate and glucagon levels in HF-O and HF-L rats, the elevated SREBP1c mRNAs suggest that neither the previously described inhibition of LXR by oleate (Ou *et al.* 2001) nor the elevation of glucagon suffices to inhibit SREBP1c transcription in chronic obese states. It seems likely that the hyperinsulinemia induced by these HF diets is at least a partial cause for the SREBP1c mRNA elevation. From this and the increase in pyruvate kinase mRNA levels – a well-known transcriptional effect of insulin presumably independent of SREBP1c (Stoeckman & Towle 2002) – in the hyperinsulinemic diet groups, it must be concluded that the regulation of hepatic gene expression by insulin was sustained in the steatotic rat livers. This points to a possible dissociation of the insulin resistance phenomenon: in obesity, peripheral insensitivity to insulin-induced glucose disposal may not be necessarily connected to impairment of insulin's transcriptional effects. This could lead to a vicious cycle, as the resulting increase of lipogenesis further impairs insulin's metabolic actions.

Although lipogenesis was preferentially induced by HF-L, HF-O and HF-C, some PPARα-regulated genes, such as the fatty acid translocase CD36 (Bonen *et al.* 2004), were also upregulated in these rats. This can be explained by the hepatic PPARα activation induced by saturated and monounsaturated fatty acids, as recently reviewed by (Duplus *et al.* 2000). Obviously, the

repartitioning of fatty acids away from triglyceride synthesis and toward mitochondrial oxidation (for review, see Jump 2002) by this mechanism was less potent in these diet groups than the increase of SREBP1c-mediated liposynthesis, resulting in hepatic fat accumulation and hypertriglyceridemia.

PUFA are more potent activators of PPARα than SFA or MUFA (Duplus *et al.* 2000), and consequently the PPARα-dependent genes of the fat oxidation cluster were strongly activated in fish oil-fed high fat rats. In contrast to the previously described downregulation of SREBP1c mRNA by fish oil feeding (Xu *et al.* 1999) and despite relatively low insulin levels, the SREBP1c gene expression was moderately upregulated in HF-F rats in our model. This might be explained by differences in ω-3 PUFA plasma levels or alterations caused by the longer diet regime in our study as well as by rat / mouse species differences. Looking at the ratio between PPARα-activated and SREBP1c-activated genes in HF-F rats, it is probable that the fish oil-induced activation of PPARα overrides the SREBP1c-mediated liposynthetic effects. Moreover, it has been described that ω-3 PUFA decrease the levels of mature nuclear SREBP1c *protein* (Worgall *et al.* 1998, Nakatani *et al.* 2003), an effect that also can explain a net decrease of liposynthetic gene expression in the HF-F diet group.

In conclusion, recapitulating the phenotypical and metabolic data we obtained, lard certainly can be recommended as one of the standard fats to be used for the generation of a valid rat model for the metabolic changes associated with obesity. In Wistar rats, a high olive oil intake does not protect from high fat induced metabolic changes. High coconut fat consumption appears to be less deleterious in terms of obesity and insulin resistance, but it is associated with prominent hepatic steatosis and hypertriglyceridemia. These effects are accompanied by an upregulation of SREBP1c and liposynthetic genes. Animals fed with high fat diets based on fish oil remain lean and insulin-sensitive; based on liver gene profiling this is due to a probably PPARα-mediated predominant induction of the fat oxidation gene cluster. From our data it is conceivable that a dissociation between the peripheral and / or hepatic resistance to the metabolic insulin effects and sustained sensitivity to the transcriptional insulin effects is a major aspect in the pathogenesis of obesity-related metabolic disorders.

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