Dietary soya protein concentrate enriched with isoflavones reduced fatty liver, increased hepatic fatty acid oxidation and decreased the hepatic mRNA level of VLDL receptor in obese Zucker rats

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Dietary soya protein concentrate enriched with isoflavones were fed to obese Zucker rats for 6 weeks. HDI feeding, but not LDI feeding, reduced the fatty liver and decreased the plasma levels of alanine transaminase and aspartate transaminase. This was accompanied by increased activities of mitochondrial and peroxisomal β-oxidation, acetyl-CoA carboxylase, fatty acid synthase and glycerol-3-phosphate acyltransferase in liver and increased triacylglycerol level in plasma. The decreased fatty liver and the increased plasma triacylglycerol level appeared not to be caused by an increased secretion of VLDL, as HDI decreased the hepatic mRNA levels of apo B and arylacetamide deacetylase. However, the gene expression of VLDL receptor was markedly decreased in liver, but unchanged in epididymal white adipose tissue and skeletal muscle of rats fed HDI, indicating that the liver may be the key organ for the reduced clearance of triacylglycerol-rich lipoproteins from plasma after HDI feeding. The n-3/n-6, 20:4n-6/18:2n-6 and (20:5n-3 + 22:6n-3)/18:3n-3 ratios were increased in liver triacylglycerol by HDI. The phospholipids in liver of rats fed HDI contained a low level of 20:4n-6 and a high level of 20:5n-3, favouring the production of anti-inflammatory eicosanoids. When obese Zucker rats were fed soya protein, this also resulted in reduced fatty liver, possibly through reduced clearance of VLDL by the liver. We conclude that the isoflavone-enriched soya concentrate as well as soya protein may be promising dietary supplements for treatment of non-alcoholic fatty liver.

Steatosis: β-Oxidation: Lipogenesis

Non-alcoholic fatty liver disease increases 10–24% of the general global population, but the prevalence significantly increases, to more than 50%, for obese people (Silverman et al. 1990; Angulo & Lindor, 2002). It has been estimated that 5–10% of obese individuals are obese due to insufficient leptin production, while the remaining 90–95% are believed to be leptin resistant (Cohen & Friedman, 2004). The genetically obese Zucker fa/fa rats have a null mutation of the leptin receptor gene and have been extensively studied as a model for obesity in man. These rats also have high plasma triacylglycerol levels and abnormally high synthesis of triacylglycerol in liver, combined with a low rate of fatty acid oxidation in liver (Bray, 1977; Triscari et al., 1982), leading to development of fatty liver at a young age (Krief & Bazin, 1991). The obese Zucker fa/fa rats may therefore be useful as an animal model for a deeper understanding of biochemical changes that occur during the development and treatment of fatty liver.

There is no widely accepted therapy for fatty liver, but lifestyle modifications with weight reduction are frequently recommended (McClain et al. 2004), and one approach has been changes in the diet with special focus on dietary fats. Increasing evidence suggests that not only dietary lipids and fatty acids, but also proteins and amino acids in the diet can affect lipid metabolism. It has been known for some years that dietary soya products lower the serum total cholesterol and LDL cholesterol in man (Anderson et al. 1995) and animals (Sirtori et al. 1993), and may have a cardioprotective effect. The components and mechanisms responsible for the hypcholesterolaemic effect of soya products have not been clarified. It has been suggested that the amino acid composition of soya protein, with low ratios of methionine/glycine and lysine/arginine, contribute to its cholesterol-lowering effect (Kritchevsky et al. 1982; Morita et al. 1997), but several reports have shown that this might be due to components other than amino acids, e.g. isoflavones (primarily genistein and daidzein) that are associated with the soya protein (Huff et al. 1977; Potter, 1995; Balmir et al. 1996; Madani et al. 1998; Peluso et al. 2000; Fukui et al. 2002; Ali et al. 2004).

Abbreviations: AADA, arylacetamide deacetylase; CPT, carnitine palmitoyltransferase; FAT/CD36, fatty acid translocase; HDI, diet containing a high dose of soya protein concentrate enriched with isoflavones; LDI, diet containing a low dose of soya protein concentrate enriched with isoflavones; LPL, lipoprotein lipase; SCD-1, stearoyl-CoA desaturase; WAT, white adipose tissue.

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We have previously demonstrated that when obese Zucker rats were fed a diet containing soya protein, which contains isoflavones, a lowering of the plasma cholesterol level and of the lipid content in liver resulted (Wergedahl et al. 2004). Similar results have also been reported by Peluso et al. (2000) after dietary treatment with the isoflavone-enriched fraction of isolated soya protein to obese Zucker rats. In the present study we performed two experiments: first, we wanted to study whether a dietary soya protein concentrate enriched in isoflavones could reduce the fatty liver in obese Zucker rats, and to elucidate the mechanism behind this event; second, obese Zucker rats were fed intact soya protein to see whether this would reduce the fatty liver via the same mechanisms as the isoflavone-enriched soya concentrate.

Materials and methods

**Animals and diets**

Two experiments were performed. In the first experiment, male obese Zucker rats Crl/ZUC/FaBR (fa/fa) from Charles River Laboratory, (Sulzfeld, Germany), weighing 80–135 g, were divided into three experimental groups of six rats each with comparable mean body weight. The rats were housed individually all through the feeding experiment in a room maintained at a 12 h light–dark cycle and a constant temperature of 20°C and a relative humidity of 65 ± 15%. The rats were acclimatised under these conditions before the start of the experiment. The rats were fed diets consisting of 20% (by weight) protein from casein sodium salt from bovine milk (C-8654; Sigma-Aldrich Norway AS, Oslo, Norway) or casein-added fermented soyabeans with high contents of genistein and daidzein. Two doses of isoflavones were used. The diet with a low amount of soya protein concentrate enriched with isoflavone (LDI) contained 21.4% casein and 0.7% fermented soya proteins, providing 0.40 g genistein/kg diet and 0.45 g daidzein/kg diet. The diet with a high-dose of soya protein concentrate enriched with isoflavone (HDI) contained 18.4% casein and 6.7% fermented soya proteins, providing 4.00 g genistein/kg diet and 4.50 g daidzein/kg diet. Choline hydrogentartrate (0.2% Merck, Darmstadt, Germany) was added to all diets. The rats were fed these diets for 6 weeks. All rats had free access to tap water and feed. Based on daily observations of the rats, it seemed that all experimental diets were well tolerated. Table 1 gives an overview of the experimental diets.

In the second experiment, male obese Zucker rats Crl/ZUC/FaBR (fa/fa) from Charles River Laboratory, weighing 80–135 g, were fed diets similar to the diets in the first experiment, except that they contained 20% protein from either soya protein or casein, and the rats were fed these diets for 3 weeks. Table 1 gives an overview of the experimental diets (for more details on the diets and experimental conditions, see Wergedahl et al. 2004).

At the end of the feeding period, under non-fasting conditions, the rats were anaesthetised with a 1:1 mixture of Hypnorm™ (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Pharmaceutica, Beersse, Belgium) and Dormicum® (5 mg/ml midazolam; F. Hoffmann-La Roche AG, Basel, Switzerland) injected subcutaneously. Blood was drawn directly from the heart using a syringe containing heparin. The liver was immediately removed, weighed and divided into two parts, which were

<table>
<thead>
<tr>
<th>Table 1. Composition of the experimental diets (g/kg diet)*</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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</table>

*The diets were isoenergetic and isonitrogenous, and contained 200 g crude protein/kg diet.
† Casein (g/kg diet): fat (98), ash (31). The casein protein contained 91.9% crude protein.
‡ LDI, diet containing a low dose of soya protein concentrate enriched with isoflavones (g/kg diet): fat (99), genistein (0.40), daidzein (0.45). The soy protein concentrate contained 45.4% crude protein
§ HDI, diet containing a high dose of soya protein concentrate enriched with isoflavones (g/kg diet): fat (99), genistein (4.00), daidzein (4.50). The soy protein concentrate contained 45.4% crude protein.
||Diet containing soya protein (g/kg diet): fat (101), ash (54). The soy protein contained 88.0% crude protein. The diets in Experiment 2 had 2% NaCl added.
† Fatty acid composition of the soya oil (mean of two measurements, deviation less than 3%, shown as g/100 g fat): 18:2-6 (55.9), 18:1-9 (21.4), 16:0 (11.4), 18:3n-3 (5.8), 18:0 (3.3), 18:1n-7 (1.6).
** AIN-93G-MX (Dyets Inc., Bethlehem, PA, USA).
†† AIN-93VX (Dyets Inc., Bethlehem, PA, USA).

Fatty acid composition

Lipids were extracted from liver and plasma samples using a mixture of chloroform and methanol (Bligh & Dyer, 1959). The lipid classes in liver were separated by TLC on silica gel plates (0.25 mm Silica gel 60; Merck, Darmstadt, Germany) was added to all diets. The rats were fed these diets for 6 weeks. All rats had free access to tap water and feed. Based on daily observations of the rats, it seemed that all experimental diets were well tolerated. Table 1 gives an overview of the experimental diets (for more details on the diets and experimental conditions, see Wergedahl et al. 2004).

At the end of the feeding period, under non-fasting conditions, the rats were anaesthetised with a 1:1 mixture of Hypnorm™ (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Pharmaceutica, Beersse, Belgium) and Dormicum® (5 mg/ml midazolam; F. Hoffmann-La Roche AG, Basel, Switzerland) injected subcutaneously. Blood was drawn directly from the heart using a syringe containing heparin. The liver was immediately removed, weighed and divided into two parts, which were immediately chilled on ice or frozen in liquid N. Plasma, liver, epididymal white adipose tissue (WAT) and skeletal muscle from the thigh were stored at −80°C until analysis. The protocol was approved by the Norwegian State Board of Biological Experiments with Living Animals.

**Scharlach red staining of liver**

In order to study neutral fat deposits, 10 μm frozen sections from the livers were cut, stained with a filtered Scharlach red solution and visualised by light microscopy.
Lipid quantification

Triacylglycerol in liver, plasma and triacylglycerol-rich lipoproteins were measured enzymatically using the triacylglycerol kit from Bayer (Tarrytown, NY, USA). Phospholipids in liver were measured using the phospholipid kit from BioMérieux (Lyon, France). NEFA in plasma were measured using the NEFA C kit from Wako Chemicals (Dalton, OH, USA) on the Hitachi 917 system (Roche Diagnostics GmbH, Mannheim, Germany). Lipid extracts were prepared as previously described (Muna et al. 2002).

Plasma transaminases

The plasma levels of alanine transaminase and aspartate transaminase were measured on the Hitachi 917 system (Roche Diagnostics) using the appropriate kits from Roche Diagnostics.

Real-time quantitative RT–PCR

Total RNA was purified from frozen liver, epididymal WAT and skeletal muscle using RNeasy Midi Kit (Qiagen, Hilden, Germany). For isolation of RNA from WAT, QIAzol Lysis Reagent (Qiagen) was added to the samples and the extraction was performed with chloroform. For isolation of RNA from muscle, proteinase K (Qiagen) was added to the samples. Primers and Taqman probe for rat carnitine palmitoyltransferase (CPT)-Ia, CPT-II, Δ5 desaturase, Δ6 desaturase, glyceraldehyde-3-phosphate dehydrogenase, PPARα, stearoyl-CoA desaturase and sterol regulatory binding protein-1c were designed using Primer Express (Applied Biosystems, Foster City, CA, USA). Gene expressions were determined using Applied Biosystems, Foster City, CA, USA. Gene expressions were determined using Taqman probes or SYBRgreen. The following sequences were used: CPT-Ia forward 5'-CCC AGT GGC GAC TCT T T-3' and reverse 5'-TGC ATT GAG GTA TCT CTT CAT GGT-3', probe 5'-TGC CCA GGC TGC CTA-3', Δ5 desaturase forward 5'-TTG ATG GTA TCT CCT CAT GGT-3' and probe 5'-TTG GCA GGC TGC CTA-3', Δ6 desaturase forward 5'-CAG CCG GCA CCT CAA TTT-3' and reverse 5'-TTG TTT GCC GAG AGA TCT-3' and probe 5'-CAG ATT GAC CAC CTC TTC CCC AC-3', glyceraldehyde-3-phosphate dehydrogenase forward 5'-TTG ACC ACC AAC TGC TTA GC-3', reverse 5'-CAG TCT GAG TGG CAG TGA TG-3' and probe 5'-TGG AAG GGC TCA TGA CCA CAG TCC A-3', PPARα forward 5'-CAG ATG CCA CGC ATG TGA AG-3', reverse 5'-TGG CAG TCT TCT TGT TCAG AGA CAC CT-3' and probe 5'-CAG TTC AGG CCC AGG C-3', Δ6 desaturase forward 5'-CAG CCG GCC CCA CCT CAA TTT-3' and reverse 5'-TTG TTT GCC GAG AGA TCT-3' and probe 5'-CAG ATT GAC CAC CTC TTC CCC AC-3', glyceraldehyde-3-phosphate dehydrogenase forward 5'-TTG ACC ACC AAC TGC TTA GC-3', reverse 5'-CAG TCT GAG TGG CAG TGA TG-3' and probe 5'-TGG AAG GGC TCA TGA CCA CAG TCC A-3', PPARα forward 5'-CAG ATG CCA CGC ATG TGA AG-3', reverse 5'-TGG CAG TCT TCT TGT TCAG AGA CAC CT-3' and probe 5'-CAG TTC AGG CCC AGG C-3'.

Preparation of hepatic subcellular fractions

Homogenisation and subcellular fractionation of the livers were performed as previously described (Berge et al. 1984). The procedure was performed at 0–4°C, and the fractions were stored at −80°C. Protein was assayed with the BioRad protein assay kit (BioRad, Richmond, CA, USA) using bovine serum albumin as the standard.

Enzyme assays

Palmitoyl-CoA and palmitoyl-carnitine oxidation were measured in the mitochondrial fraction as acid-soluble products (Willumsen et al. 1993). CPT-I and CPT-II activities were measured in the mitochondrial fraction (Madsen et al. 1998). Fatty acyl-CoA oxidase was measured in the peroxisomal fraction (Small et al. 1985). 3-Hydroxy-3-methylglutaryl-CoA synthase was measured spectrophotometrically in the mitochondrial fraction (Clinkenbeard et al. 1975). Acetyl-CoA carboxylase activity was measured in cytosolic fraction by measuring the amount of NaH14CO3 incorporated into malonyl-CoA (Tanabe et al. 1981). Fatty acid synthase activity was measured in the cytosolic fraction as described by Roncari et al. (1981), modified according to Skorve et al. (1993). Glycerol-3-phosphate acyl transferase activity was measured in mitochondrial and microsomal fractions as described by Bates & Saggerson (1977). Acyl-CoA synthetase activity was measured in the mitochondrial fraction (Gudbrandsen et al. 2005).

Malonyl-CoA

Malonyl-CoA in the liver was measured by reversed-phase HPLC (Wergedahl et al. 2004).

Isolation of triacylglycerol-rich lipoproteins

Plasma from two rats was pooled to obtain a volume of 3 ml. The plasma triacylglycerol-rich lipoprotein fraction was prepared as previously described (Muna et al. 2002).
Statistical analysis

All data in the tables are presented as means and standard deviations for six rats per group. The data were evaluated by one-way ANOVA and Dunnett’s test (Experiment 1, when three feeding groups were included) or by unpaired Student’s *t* test (Experiment 1, when two feeding groups were included, and Experiment 2) with the level of statistical significance set at *P*<0.05 (GraphPad Prism, version 3.0; GraphPad Prism, San Diego, CA, USA). Rats fed casein-based diet served as controls.

Results

Hepatic lipids and fatty acid composition

Scharlach red staining of the livers demonstrated that the levels of neutral lipids were decreased by HDI feeding, as the lipid droplets were both fewer and smaller (Fig. 1). Quantification of the hepatic triacylglycerol level showed that HDI reduced the level of triacylglycerol by 67% (Table 2). The reduced fatty liver by HDI was accompanied with reduced plasma levels of alanine transaminase and aspartate transaminase by more than 80% (Table 2). The hepatic phospholipid level was not changed after HDI feeding (Table 2). When rats were fed LDI, no effects were seen in the liver lipid content or in the plasma transaminases (Fig. 1; Table 2).

HDI feeding changed the fatty acid composition of the hepatic lipids (Table 3). The level of 16:0 was decreased in triacylglycerol, phospholipids and 1,2-diacylglycerol, whereas the level of 18:0 was increased in phospholipids and 1,2-diacylglycerol after HDI feeding (Table 2). This resulted in an increased 18:0/16:0 ratio in phospholipids, triacylglycerol and 1,2-diacylglycerol (calculated from Table 3), concomitant with an increased mRNA level of elongase in liver of HDI-fed rats (Table 4).

The levels of the Δ9 desaturated fatty acids 16:1n-7 and 18:1n-9 in liver lipids were not changed by HDI feeding, except for a decreased level of 18:1n-9 in 1,2-diacylglycerol (Table 3). The mRNA level of stearoyl-CoA desaturase-1, the rate-limiting enzyme catalysing the synthesis of Δ9 desaturated MUFA, was not significantly changed by HDI (Table 4).

The phospholipid levels of 18:2n-6, 18:3n-6, 20:3n-6, 18:3n-3 and 20:5n-3 were increased and the level of 20:4n-6 was decreased after HDI feeding. In addition, HDI-fed rats had an increased level of 20:3n-6 in triacylglycerol and 1,2-diacylglycerol and of 20:4n-6 acid in triacylglycerol, 1,2-diacylglycerol and as NEFA. HDI feeding also increased the level of 20:5n-3 in 1,2-diacylglycerol, and that of 22:5n-3 and 22:6n-3 in triacylglycerol (Table 3). The ratio of n-3 to n-6 was increased in triacylglycerol and decreased in 1,2-diacylglycerol by HDI feeding (Table 5). HDI feeding decreased the 20:4n-6/18:2n-6 and the (20:5n-3 + 22:6n-3)/18:3n-3 ratios in phospholipids, whereas these ratios were increased in triacylglycerol (Table 5). The Δ6 and Δ5 desaturases catalyse the biosynthesis of long-chain n-6 and n-3 PUFA from the precursors 18:2n-6 and 18:3n-3, respectively. The mRNA level of Δ6 desaturase was not affected, but the mRNA level of Δ5 desaturase was significantly decreased by HDI feeding (Table 4).

The fatty acid composition of phospholipids, triacylglycerol, 1,2-diacylglycerol and NEFA and the mRNA levels of elongase and desaturases in liver were not affected by LDI feeding (data not shown).

Fig. 1. Fat-stained microphotographs from livers of Zucker rats fed a diet containing casein, a low dose of soya protein concentrate enriched with isoflavones (LDI), a high dose of soya protein concentrate enriched with isoflavones (HDI) or soya protein, showing one representative example from each feeding group. For details of procedures, see p. 250. Scharlach red, original magnifications × 50.
and the shorter n-3 PUFA were similar in plasma and hepatic phospholipids after HDI feeding (Table 3). HDI feeding increased the levels of 16:0, 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6, 18:3n-3, 20:5n-3 and 22:5n-3 and reduced the levels of 20:4n-6 and 22:6n-3 (Table 3).

**Secretion and clearance of lipids**

HDI feeding decreased the mRNA levels of both AADA and apo B (Table 4). The mRNA level of hormone-sensitive lipase in epididymal WAT, and that of VLDL receptor and LPL in epididymal WAT and skeletal muscle were not changed (Table 8). HDI feeding increased the mRNA level of LDL receptor in epididymal WAT and skeletal muscle by 86 and 37 %, respectively (Table 8). The mRNA level of FAT/CD36 was increased by 60 % in epididymal WAT (Table 8), but was not changed in liver (Table 4) or in skeletal muscle (Table 8). The gene expressions of VLDL receptor and LPL in liver were down-regulated by 74 and 93 %, respectively, in rats fed HDI (Table 4).

**Soya protein experiment**

When obese Zucker rats were fed intact soya protein, a distinct decrease was seen in the size and number of fat droplets in liver as compared with casein-fed rats (Fig. 1). Soya protein feeding had no effect on the gene expressions of apo B and AADA, but down-regulated the mRNA levels of VLDL receptor and LPL in liver by almost 70 % (Table 9).

**Discussion**

When obese Zucker rats were fed a diet containing casein with a high amount of an isoflavone-enriched soya concentrate added (HDI), fewer and smaller fat droplets in liver resulted when compared with control rats fed casein. Quantitative measurements showed that the level of triacylglycerol in the liver was reduced accordingly. The reduced plasma levels of transaminases further supported that HDI feeding reduced fatty liver in this animal model.

The reduced fatty liver by HDI could be caused by increased β-oxidation, increased lipid biosynthesis, increased secretion of VLDL and/or decreased clearance from plasma. The increased β-oxidation after HDI feeding reduced the availability of fatty acids for biosynthesis and secretion of triacylglycerol as VLDL. However, the increased lipogenesis and glycerolipid biosynthesis suggested that increased amounts of triacylglycerols and phospholipids were available for VLDL secretion. The decreased mRNA levels of AADA, a lipase involved in the mobilisation of triacylglycerol from hepatocytes to the VLDL particle (Trickett et al. 2001), and of apo B, provided strong indices of a reduced secretion of VLDL from the liver of HDI-fed rats. Thus, these data argue against an increased hepatic secretion of VLDL particles as the cause for the reduced fatty liver in HDI-fed rats.

The markedly down-regulated gene expression of the VLDL receptor and of LPL in liver by HDI may explain both the reduced fatty liver and the increased contents of triacylglycerol and NEFA in plasma. Normally, VLDL receptor and LPL are mainly expressed in adipose tissue and muscle, and only at very low levels in liver (Oka et al. 1994). As
Table 3. Selected fatty acids (shown as g/100 g fatty acids) in liver and plasma of obese Zucker rats fed a diet containing casein or a high dose of soya protein concentrate enriched with isoflavones (HDI)†
(Mean values and standard deviations for six rats per group)

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<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Casein</td>
<td>17·5</td>
<td>1·0</td>
<td>12·6</td>
<td>0·7</td>
<td>1·1</td>
<td>0·9</td>
<td>6·4</td>
<td>1·2</td>
<td>11·9</td>
<td>1·2</td>
<td>0·40</td>
</tr>
<tr>
<td>HDI</td>
<td>24·0*</td>
<td>2·7</td>
<td>11·0</td>
<td>2·7</td>
<td>32</td>
<td>2·5</td>
<td>19·5*</td>
<td>2·7</td>
<td>16·0*</td>
<td>1·9</td>
<td>0·51*</td>
</tr>
</tbody>
</table>

ND, not detected.
Mean values in a column for phospholipids, triacylglycerol, 1,2-diacylglycerol, NEFA or plasma were significantly different from those of the control (casein) group: *P<0.05.
†For details of diets and procedures, see Table 1 and p. 250.
HDI did not affect the mRNA levels of these genes in epididymal WAT or skeletal muscle, the liver appears to be the key organ for the reduced clearance of VLDL from plasma. Furthermore, the unchanged gene expression of LPL in liver (Gudbrandsen et al. 2005) supports the hypothesis that HDI feeding increased the triacylglycerol level both in plasma and triacylglycerol-rich lipoproteins, the LDL receptor appears to contribute little to the uptake of VLDL in these rats. The increased plasma level of NEFA after HDI feeding was probably not due to an increased release from epididymal WAT to plasma, as the mRNA level of hormone-sensitive lipase implied that the biosynthesis of long-chain PUFA, especially the n-3/fatty acids and triacylglycerol, as long-chain PUFA, especially the n-3 fatty acids and triacylglycerol, is support the hypothesis that HDI feeding increased the triacylglycerol level both in plasma and triacylglycerol-rich lipoproteins, the LDL receptor appears to contribute little to the uptake of VLDL in these rats. The increased plasma level of NEFA after HDI feeding was probably not due to an increased release from epididymal WAT to plasma, as the mRNA level of hormone-sensitive lipase implied that the biosynthesis of fatty acids and triacylglycerol, as long-chain PUFA, especially the n-3 fatty acids and triacylglycerol, is support the hypothesis that HDI feeding increased the triacylglycerol level both in plasma and triacylglycerol-rich lipoproteins, the LDL receptor appears to contribute little to the uptake of VLDL in these rats. The increased plasma level of NEFA after HDI feeding was probably not due to an increased release from epididymal WAT to plasma, as the mRNA level of hormone-sensitive lipase implied that the biosynthesis of fatty acids and triacylglycerol, as long-chain PUFA, especially the n-3 fatty acids and triacylglycerol, is support the hypothesis that HDI feeding increased the triacylglycerol level both in plasma and triacylglycerol-rich lipoproteins, the LDL receptor appears to contribute little to the uptake of VLDL in these rats. The increased plasma level of NEFA after HDI feeding was probably not due to an increased release from epididymal WAT to plasma, as the mRNA level of hormone-sensitive lipase implied that the biosynthesis of fatty acids and triacylglycerol, as long-chain PUFA, especially the n-3 fatty acids and triacylglycerol, is support the hypothesis that HDI feeding increased the triacylglycerol level both in plasma and triacylglycerol-rich lipoproteins, the LDL receptor appears to contribute little to the uptake of VLDL in these rats. The increased plasma level of NEFA after HDI feeding was probably not due to an increased release from epididymal WAT to plasma, as the mRNA level of hormone-sensitive lipase implied that the biosynthesis of fatty acids and triacylglycerol, as long-chain PUFA, especially the n-3 fatty acids and triacylglycerol, is suppo...
been reported to activate PPARα in vitro (Mezei et al. 2003), we found a decreased gene expression of not only PPARα, but also of PPARδ and PPARγ, after HDI feeding. The reduced content of 20:4n-6 and the increased content of 20:5n-3 in phospholipids after HDI feeding imply that this diet may prevent the development of atherosclerosis and other inflammatory diseases, as this may favour the production of anti-inflammatory over pro-inflammatory eicosanoids.

The changes in fatty acid composition in plasma after HDI feeding was not similar to what was seen in any lipid fraction isolated from liver. Since triacylglycerol-rich lipoproteins from obese Zucker rats fed casein contain approximately 80% triacylglycerol (OA Gudbrandsen, H Wergedahl and RK Berge, unpublished results), the triacylglycerols will contribute to a majority of the fatty acids in plasma. Certain analogous changes were seen in plasma and liver phospholipids, especially regarding the n-6 and n-3 PUFA, possibly because a sizeable portion of VLDL triacylglycerol arise from cellular fatty acids esterified as phospholipids (Wiggins & Gibbons, 1996). The markedly increased 18:1n-9 and decreased 20:4n-6 levels in plasma after HDI feeding were not seen in any liver lipids except for cholesterol esters (Gudbrandsen et al. 2005), supporting findings showing that cholesterol esters of VLDL are non-selectively transferred from the liver (Gidez et al. 1965).

We have previously demonstrated that dietary soya protein lowers the hepatic lipid content in obese Zucker rats (Wergedahl et al. 2004). In the present study we show that the mechanism of action of the soya protein is similar to that of HDI, as soya protein appears to enhance β-oxidation (Wergedahl et al. 2004) and probably does not increase the secretion of VLDL from liver, as the mRNA levels of AADA and apo B were not changed. In addition, soya protein feeding decreased the mRNA levels of VLDL receptor and LPL, indicating that also with intact soya protein the reduced fatty liver might be due to decreased clearance of VLDL from plasma. Since the amino acid compositions of casein and HDI diets were almost similar (Gudbrandsen et al. 2005), while that of soya protein was markedly different (Wergedahl et al. 2004), this suggests that the isoflavones and not the amino acids may be the bioactive components of the HDI diet.

To conclude, the reduced fatty liver in obese Zucker rats fed HDI or soya protein appear to be due to increased catabolism of fatty acids in liver combined with reduced clearance of VLDL from the circulation by the liver.

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### References


