Regulatory enzymes of mitochondrial B-oxidation as targets for treatment of the metabolic syndrome
Bijker-Schreurs, Marijke

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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SORAPHEN, AN INHIBITOR OF THE ACETYL-COA CARBOXYLASE SYSTEM, IMPROVES PERIPHERAL INSULIN SENSITIVITY IN MICE FED A HIGH FAT DIET

Marijke Schreurs¹,
Theo H. van Dijk²,
Albert Gerding²,
Rick Havinga¹,
Dirk-Jan Reijngoud²,
Folkert Kuipers¹,²

¹Departments of Pediatrics and ²Laboratory Medicine, Center for Liver Digestive and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Accepted for publication in Diabetes, Obesity and Metabolism as a research report
Chapter 4

ABSTRACT

Aim: Inhibition of the acetyl-CoA carboxylase (ACC) system, consisting of the isoenzymes ACC1 and ACC2, may be beneficial for treatment of insulin resistance and/or obesity by interfering with de novo lipogenesis and β-oxidation processes. We have evaluated metabolic effects of pharmacological inhibition of ACC by Soraphen on high fat diet-induced insulin resistance in mice.

Methods: Male C57Bl6/J mice were fed either a control chow, a high fat (HF) diet or a HF diet supplemented with Soraphen (50 mg/kg/d or 100 mg/kg/d).

Results: Body weight gain and total body fat content of Soraphen-treated animals was significantly reduced compared to HF-fed mice. Hepatic triglyceride content was not affected upon treatment, while plasma triglycerides were slightly increased by Soraphen. The fractional synthesis of palmitate was significantly reduced in mice treated with Soraphen compared to controls, indicative for ACC1 inhibition. Plasma β-hydroxybutyrate levels were significantly elevated by Soraphen, reflecting simultaneous inhibition of ACC2 activity. Mice treated with Soraphen showed a dose-dependent improvement of peripheral insulin sensitivity, as assessed by hyperinsulinemic euglycemic clamps (glucose infusion rate: HF -43±11%, SP50 -15±6%, SP100 -1±9% vs chow fed mice).

Conclusions: Pharmacological inhibition of the ACC system is of potential use for treatment of key components of the metabolic syndrome.
INTRODUCTION

The acetyl-CoA carboxylase (ACC) system is involved in the regulation of both lipogenesis and mitochondrial β-oxidation. ACC catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA. Malonyl-CoA is utilized for the synthesis of fatty acids but also inhibits the action of carnitine palmitoyltransferase 1 (CPT), the key enzyme in control of mitochondrial β-oxidation. In this way, ACC contributes to control of both fatty acid synthesis and fatty acid utilization. Two isoforms of ACC have been identified that are encoded by separate genes. ACC1 (also known as ACCα) is a 265 kDalton protein located in the endoplasmic reticulum (ER) and is mainly present in “lipogenic” organs and tissues such as liver and adipose tissue. ACC2, or ACCβ, contains 114 additional amino acids at its N-terminus and is 280 kDalton in size. This extension anchors the protein onto the outer mitochondrial membrane where the generated malonyl-CoA inhibits CPT1 activity. ACC2 is highly expressed in skeletal muscle and the heart, but also in the liver.

Studies by Wakil et al. have clearly established that malonyl-CoA generated by the two isoforms fulfills different physiological actions. ACC2−/− mice showed a continuously high β-oxidation activity and an unaffected synthesis of fatty acids [1–4]. ACC1−/− mice were found to be not viable which confirms that lipogenesis is essential for early (fetal) development [5]. Experiments in heterozygous ACC1+/- mice showed a normal β-oxidation rate in these animals but reduced lipogenesis [5]. Thus, malonyl-CoA generated by the ACC1 isoform is primarily used for lipogenesis and ACC2 is actively involved in repression of mitochondrial β-oxidation. This has been confirmed by Savage et al. [6] who used an antisense oligonucleotide approach (ASO) against either ACC1 or ACC2 or against both isoforms in rats fed a high fat diet [6]. Recently, Choi et al. showed that ACC2−/− mice fed a high fat diet were protected from diet-induced insulin resistance. These animals even showed signs of improved insulin sensitivity, since fasting plasma insulin and glucose levels were reduced by about 30% [7]. The phenotype of the ACC2−/− mice supports the notion that inhibition of ACC, or more specifically of ACC2, might be a promising approach for treatment of insulin resistance [7].

Pharmacological agents for inhibition of the ACC system have been developed in the past years and are of potential use for treatment of specific components of the metabolic syndrome like insulin resistance and obesity. Harwood et al. [8–11] showed that wild-type mice treated with the general ACC inhibitor CP-640186 developed improved insulin sensitivity. However, in insulin-resistant ob/ob mice a further, unexplained deterioration of their metabolic condition was observed upon CP-640186-treatment [8].

Soraphen has been identified as a compound with inhibitory actions on both isoforms of ACC. This compound, originally isolated from the myxobacterium Sorangium cellulosum, inhibits fungal and other eukaryotic ACC systems [8,12–14] as well as mammalian ACC’s [8,14].
In this study, we have supplied Soraphen at two different doses (50 mg/kg/d and 100 mg/kg/d) to mice fed a high fat (HF) diet. Treated mice showed a remarkable deficit in body weight gain and total body fat content compared to HF-fed control mice. De novo lipogenesis in Soraphen-treated mice was clearly decreased, indicating inhibition of ACC1. Ketone body levels were elevated upon treatment with Soraphen which is indicative for functional ACC2 blockade. Hyperinsulinemic euglycemic clamp studies showed a remarkable improvement of insulin sensitivity, which was most pronounced in mice treated with the highest dose of Soraphen. Thus, this study shows that pharmacological blockade of the ACC system with Soraphen provides powerful means to reverse diet-induced obesity and insulin resistance in mice, which provides new options for development of treatment strategies against obesity-related disorders.

**MATERIAL AND METHODS**

**Animals and experimental design**

Male mice (C57Bl6/J, Charles River, l’Arbresle, France) were housed in an environmentally controlled facility and randomized in four groups. Water and food were freely accessible, and the light-dark cycle was set at 12 h light and 12 h dark (light on at 7:00 a.m.). The experiments were performed in accordance with local guidelines for the use of experimental animals. The control mice were fed a standard lab chow (C) diet (carbohydrate 52, protein 35, fat 13 energy%, Abdiets, Woerden, The Netherlands). The other three groups were either fed a high fat (HF) diet (carbohydrate 21, protein 21, fat 58 energy%, Abdiets, Woerden, The Netherlands) or the HF-diet supplemented with Soraphen (SP) (provided by Dr. Rolf Jansen, Helmholtz Centre for Infection Research, Braunschweig, Germany) in a concentration aimed at 50 mg/kg/d or 100 mg/kg/d (SP$_{50}$ and SP$_{100}$), respectively. The mice received the diet for six weeks. At the end of the dietary period, mice were subjected to in vivo measurements of glucose metabolism and substrate utilization or were sacrificed for plasma and tissue collection and carcass analysis.

**Tissue and organ collection**

Mice were sacrificed by cardiac puncture under isoflurane anesthesia after a period of 4 h of fasting to avoid postprandial effects. Liver, skeletal muscle from the limb and white adipose tissue (epidydimal fat) were removed and immediately frozen in liquid nitrogen and stored at -80 °C for biochemical analysis. Blood samples were centrifuged and plasma was removed and stored at -20 °C until analysis. The remaining of the carcass was wrapped in aluminum foil and stored at -20 °C for total fat content analysis by carcass analysis.
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Carcass analysis

Animals were dissected and the total fat content of the separate tissues was determined by drying the organs at 103 °C followed by a fat extraction procedure using petroleum ether (Boom BV, Meppel, The Netherlands) and subsequent drying at 103 °C as described by Vaanholt et al. [15].

Analyses of plasma parameters

Commercially available kits were used to determine plasma triglycerides (TG), free and total cholesterol, free fatty acids, β-hydroxybutyrate and ALAT/ASAT levels (Roche Diagnostics, Mannheim, Germany; Wako Chemicals, Neuss, Germany and Spinreact, Sant Esteve de Bas, Spain). Plasma insulin levels were determined using the mouse insulin ultrasensitive ELISA kit (Mercodia, Upsala, Sweden).

Hepatic metabolite analyses

Liver lipid extraction was performed according to the method of Bligh and Dyer [16]. The protein content of these livers was determined by the method of Lowry. Commercially available kits were used to determine hepatic concentrations of TG and total cholesterol (Roche Diagnostics, Mannheim, Germany and Wako Chemicals, Neuss, Germany). Malonyl-CoA levels were determined according to the protocol of Berge et al. [17] with minor modifications on a Nova-Pak C-18 column (Waters, Etten Leur, The Netherlands). Hepatic glycogen levels were determined as described by Keppler et al. [18].

RNA isolation and mRNA expression levels by Q-PCR

Total RNA was isolated using Tri-reagents (Sigma, Zwijndrecht, The Netherlands) according to manufacturer guidelines. The RNA concentrations were measured using the nanodrop system (ND1000, Isogen Life Science, IJsselstein, The Netherlands). cDNA was generated using 2 µg of RNA per sample using M-MLV (Sigma, Zwijndrecht, The Netherlands). Quantitative real time PCR was performed using qPCR core kit (Eurogentech, Seraing, Belgium). The results were normalized to 36B4 mRNA expression levels. The sequences of the primers can be found at the website of pediatrics (www.LabPediatricsRug.nl)

Fat absorption test

High fat-fed and Soraphen-treated (100 mg/kg/d) mice were subjected to a fat absorption test. Nine hour fasted mice were given 200 µl of olive oil by gavage. Serial blood samples were taken at indicated times for up to 105 minutes. Plasma triglyceride (TG) levels were determined in these samples using a commercially available kit.
Determination of de novo lipogenesis

Animals fed either the high fat diet or the high fat diet supplemented with the highest dose of Soraphen received drinking water containing 2 % (wt/v) [1-13C]-acetate (Isotech, Miamisburg, OH, USA) for three days. Blood spots from tail bleeding were taken twice a day (8.00 a.m. and 5.00 p.m.) for GC-MS analysis for calculating incorporation of label into blood to allow for calculation of fractional cholesterol synthesis by mass isotopomer distribution analyses (MIDA). The contribution of de novo lipogenesis reflected by [1-13C]-acetate incorporation into palmitate (C16:0) was determined by MIDA as described by Hellerstein et al. [19,20] in liver homogenates of these mice.

Hyperinsulinemic euglycemic clamp experiment

The hyperinsulinemic euglycemic clamp procedure was performed as described by van Dijk et al. and Greffhorst et al. [21,22] with minor modifications. In short the mice were provided with a permanent jugular vein catheter with a two-way entrance. Nine hours before the start of the hyperinsulinemic euglycemic clamp, food was withdrawn. The first two hours of the clamp, mice received a solution containing 2.5 mg/ml [U-13C] glucose (3% [U-13C] 99% 13C atom % excess, Cambridge Isotope Laboratories, Andover, MA, USA) per hour, for determination of basal glucose turnover. During the last four hours mice were subjected to the hyperinsulinemic euglycemic clamp. Blood glucose levels were monitored with the Lifescan glucose meter (Lifescan Benelux, Beerse, Belgium) every 15 minutes. For GC-MS analysis and the calculation of the glucose turnover rate, glucose disposal and hepatic glucose production, a bloodspot was taken every 30 minutes by tail bleeding. Calculations were performed as described by van Dijk et al. [22].

Statistics

All values represent mean ± standard error for the number of animals indicated. Statistical analyses were assessed by Kruskall-Wallis followed by Conover test for multiple comparisons. The p-value was set at p<0.05 for being significant.

RESULTS

Hepatic and plasma parameters of lipid metabolism were not affected by inhibition of acetyl-CoA carboxylase but body weights were reduced

Mice treated with Soraphen for six weeks showed a reduction in body weight gain compared to mice fed a HF-diet (Figure 1). This was the case for both treated groups, however, in animals receiving 100 mg/kg/d this effect was statistically significant (SP50 -4±6, SP100 -9±4 % vs HF mice). In the SP100 group, the effect on body weight was already evident after the first week of treatment, while it appeared only after 3
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weeks of treatment in the SP50 group. Food intake of the mice was not affected by Soraphen treatment (data not shown).

To exclude impaired intestinal fat absorption as the cause of reduced body weight gain, a fat absorption test was performed. Fat absorption in the mice fed the high fat diet and mice given Soraphen at a dose of 100 mg/kg/d was comparable, as revealed by similar increases in plasma TG levels after administration of an oral bolus of olive oil (Figure 2).

Plasma levels of cholesterol and free fatty acids were not affected during treatment with Soraphen and were elevated in all groups of HF-fed animals compared to the control mice. Plasma triglyceride levels were significantly higher in mice treated with both concentrations of Soraphen compared to control and HF-fed mice. Ketone body levels as reflected by β-hydroxybutyrate were significantly lower in the HF-fed mice compared to chow-fed controls, but back to control levels in the SP-treated groups. Plasma insulin levels were significantly higher in the HF-fed animals after a basal period of fasting compared to the other three groups (+94% vs control). Insulin
levels of the treated mice both groups were back at control levels (Table 1). The ALAT and ASAT values were somewhat higher in the treated groups, but the differences did not reach significance, indicating that liver function in Soraphen-treated animals was not disturbed (Table 1).

Table 1: Plasma metabolic parameters after 4 hours of fasting. C: control, HF: high fat, SP50: high fat supplemented with Soraphen 50 mg/kg/d and SP100: high fat supplemented with Soraphen 100 mg/kg/d. Values are represented as mean ± SEM, number of animals 5-6. * p<0.05 vs control, # p<0.05 vs high fat.

<table>
<thead>
<tr>
<th>Plasma parameters</th>
<th>C</th>
<th>HF</th>
<th>SP50</th>
<th>SP100</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol/l)</td>
<td>0.64±0.07</td>
<td>0.96±0.14</td>
<td>1.56±0.06*</td>
<td>1.32±0.19*</td>
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<tr>
<td>Total cholesterol</td>
<td>2.51±0.15</td>
<td>4.33±0.27*</td>
<td>4.22±0.47*</td>
<td>3.43±0.32*</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.97±0.05</td>
<td>0.85±0.07</td>
<td>1.17±0.10</td>
<td>0.97±0.10</td>
</tr>
<tr>
<td>BHB (mmol/l)</td>
<td>0.23±0.03</td>
<td>0.13±0.01*</td>
<td>0.20±0.02#</td>
<td>0.23±0.03#</td>
</tr>
<tr>
<td>Insulin (µg/l)</td>
<td>0.35±0.03</td>
<td>0.68±0.21*</td>
<td>0.26±0.05#</td>
<td>0.39±0.13#</td>
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<tr>
<td>ASAT (U/l)</td>
<td>8.37±1.17</td>
<td>10.30±2.08</td>
<td>12.73±1.70</td>
<td>9.18±1.07</td>
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<tr>
<td>ALAT (U/l)</td>
<td>3.41±0.94</td>
<td>4.45±0.65</td>
<td>8.50±1.03</td>
<td>5.74±0.76</td>
</tr>
</tbody>
</table>

Table 2: Hepatic metabolic parameters after 4 hours of fasting. C: control, HF: high fat, SP50: high fat supplemented with Soraphen 50 mg/kg/d and SP100: high fat supplemented with Soraphen 100 mg/kg/d. Values are represented as mean ± SEM, number of animals 5-6. * p<0.05 vs control.

<table>
<thead>
<tr>
<th>Liver parameters</th>
<th>C</th>
<th>HF</th>
<th>SP50</th>
<th>SP100</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (µmol/g liver)</td>
<td>9.6±0.76</td>
<td>13.9±1.84</td>
<td>12.3±2.29</td>
<td>13.4±1.99</td>
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<tr>
<td>Total cholesterol (µmol/g liver)</td>
<td>3.06±0.49</td>
<td>2.47±0.11</td>
<td>2.26±0.36</td>
<td>2.37±0.45</td>
</tr>
<tr>
<td>Glycogen (µmol/g liver)</td>
<td>259.9±9.5</td>
<td>334.1±18.5</td>
<td>379.5±40.8</td>
<td>289.3±47.4</td>
</tr>
<tr>
<td>Protein (mg/g liver)</td>
<td>242±19.6</td>
<td>187±19.2</td>
<td>205±21.3</td>
<td>241±22.3</td>
</tr>
<tr>
<td>Malonyl-CoA (nmol/g liver)</td>
<td>35±3</td>
<td>28±1*</td>
<td>27±2*</td>
<td>30±2</td>
</tr>
</tbody>
</table>
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Hepatic metabolic parameters determined after 4 hrs of fasting, i.e., total cholesterol, triglyceride and glycogen contents of the three groups of HF-fed mice did not differ, also not when compared to the chow-fed animals. Hepatic malonyl-CoA levels were somewhat lower in the HF-fed mice and the SP50 mice compared to chow-fed animals (Table 2). Hepatic malonyl-CoA levels in SP100 mice were not different from those in control mice (Table 2).

**Carcass analysis revealed that the total fat content of the animals treated with Soraphen was reduced to control levels**

The total amount of fat in the HF-animals was significantly higher compared to that of the chow-fed control mice (9.75 % of total BW for control vs 18.35 % of total BW for HF). The SP50 group showed a reduction compared to the HF-fed mice in total body fat content. This effect was even more pronounced in the SP100 group: these mice had a similar amount of total body fat as the control mice did (Table 3).

The visceral fat content of all three HF-groups was higher compared to the control mice, however, a tendency towards normalized levels was seen in the SP-treated animals which was significant for mice treated with the highest dose of Soraphen.

The other fat pads analyzed were increased in the HF-group and reduced in the SP-treated groups, with most pronounced effects in the animals that received the highest dose of Soraphen (Table 3).

**Hepatic gene expression levels of genes involved in both glucose and lipid metabolism were not altered during treatment with Soraphen**

Hepatic mRNA levels were analyzed by Q-PCR to gain insight in expression patterns of genes involved in both lipid and glucose metabolism. Lipogenic genes like acetyl-CoA carboxylase (Acc1), malonyl-CoA decarboxylase (Mcd), fatty acid synthase (Fas) were not affected, with the exception of Fas in all three HF-fed groups. These expression levels were at least 3.5 fold higher compared to the control mice (Figure 3). Likewise, Acc2 mRNA levels were not affected by HF feeding or Soraphen treatment.

Genes encoding key enzymes of glucose metabolism, including glucokinase (Gk), glycogen phosphorylase (Gp), glycogen synthase (Gs) and glucose-6-phosphatase translocase and hydrolase (G6pt, G6ph) were not affected upon treatment with Soraphen (Figure 3). Gk expression levels, however, were increased during HF feeding alone, but reduced to control levels after treatment with Soraphen.

Expression levels of genes involved in lipolysis and inflammation were not affected in livers of the treated animals compared to the control animals. Tnfa levels were slightly induced in the HF-mice, but back to control levels in the Soraphen mice indicating that there was no indication for inflammation in the treated animals (Figure 3).
Gene expression patterns in skeletal muscle (Figure 4A) and in white adipose tissue (WAT) (Figure 4B) were not altered upon either high fat feeding or treatment with Soraphen compared to chow-fed controls. Only Glut1 expression levels in WAT tended to be increased in the SP50 animals.

**De novo lipogenesis is suppressed in Soraphen-treated mice**

Both de novo lipogenesis and cholesterol synthesis were measured after 2 weeks of treatment with Soraphen by supplying animals with 2% (w/v) labeled [1-13C]-

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**Table 3: Carcass analysis after Soraphen-treatment.** C: control, HF: high fat, SP50: high fat supplemented with Soraphen 50 mg/kg/d and SP100: high fat supplemented with Soraphen 100 mg/kg/d. Values are represented as mean ± SEM, number of animals 3. * p<0.05 vs control, # p<0.05 vs high fat.

<table>
<thead>
<tr>
<th>Carcass analysis</th>
<th>C</th>
<th>HF</th>
<th>SP50</th>
<th>SP100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral fat (% of tot BW)</td>
<td>1.77±0.02</td>
<td>4.37±0.04*</td>
<td>3.23±0.1</td>
<td>2.13±0.02#</td>
</tr>
<tr>
<td>Subcutaneous fat (% of tot BW)</td>
<td>3.78±0.03</td>
<td>7.79±0.05*</td>
<td>4.95±0.1#</td>
<td>3.95±0.03#</td>
</tr>
<tr>
<td>Epidydimal fat (% of tot BW)</td>
<td>0.76±0.01</td>
<td>2.31±0.03*</td>
<td>1.78±0.1</td>
<td>1.16±0.01#</td>
</tr>
<tr>
<td>Mesenchymal fat (% of tot BW)</td>
<td>0.60±0.01</td>
<td>1.13±0.0*</td>
<td>0.90±0.02</td>
<td>0.62±0.01#</td>
</tr>
<tr>
<td>Retroperitoneal fat (% of tot BW)</td>
<td>0.41±0.01</td>
<td>0.93±0.03*</td>
<td>0.55±0.1</td>
<td>0.36±0.01#</td>
</tr>
<tr>
<td>Total fat (% of tot BW)</td>
<td>9.75±0.1</td>
<td>18.35±0.1*</td>
<td>12.96±0.2#</td>
<td>9.99±0.1#</td>
</tr>
</tbody>
</table>

**Figure 3: Hepatic gene expression levels of genes involved in glucose and lipid metabolism.** Values are mean ± SEM (n=6). * p<0.05 vs control

Gene expression patterns in skeletal muscle (Figure 4A) and in white adipose tissue (WAT) (Figure 4B) were not altered upon either high fat feeding or treatment with Soraphen compared to chow-fed controls. Only Glut1 expression levels in WAT tended to be increased in the SP50 animals.
acetate in their drinking water for 3 days. Mice fed the high fat diet supplemented with Soraphen (SP_{100}) showed a small decrease in precursor-pool enrichment of the acetyl-CoA pool used for the synthesis of palmitate (C16:0) at 72 hrs in the liver (Figure 5A). The precursor-pool enrichment of newly synthesized cholesterol present in plasma was not affected (Figure 5A). The fractional synthesis of palmitate in the liver was reduced to 30% of the control value after treatment with Soraphen compared to the HF diet only. These results thus demonstrate a significant reduction of de novo lipogenesis upon ACC inhibition. The fraction of newly synthesized plasma cholesterol was not altered upon Soraphen treatment (Figure 5B).

**Inhibition of acetyl-CoA carboxylase by Soraphen improves insulin sensitivity in mice fed a high fat diet**

Blood glucose levels in the fed state were not different between the groups (data not shown). Also after a 9 h period of fasting, blood glucose levels were similar in all groups. During the first two hours of the experiment (basal period), the HF-fed group and the SP50 group showed somewhat elevated glucose levels compared to
the control mice. This was not the case for the mice treated with the highest dose of Soraphen. During the hyperinsulinemic euglycemic clamp period, glucose levels were similar except for the SP100 mice; these levels were slightly lower compared to the other three groups (Figure 6). The glucose infusion rate (GIR), required to maintain euglycemia was significantly lower in HF-fed mice than in the chow-fed controls, indicative for diet-induced insulin resistance. The GIR in the two SP-treated groups was significantly improved compared to the HF-mice. This effect was more pronounced in the SP100 group than in the SP50 group (Figure 7A). The difference between the two SP-treated groups did not reach statistical significance. The insulin-stimulated glucose disposal rate (Rd) was improved in the SP-treated groups during the clamp. A dose-dependency similar to that observed for the GIR was also seen for Rd: the difference between the two Soraphen groups was statistically significant.

Figure 5: Precursor pool enrichment (Figure A) and fractional synthesis rate (Figure B) mice fed a high fat diet and SP100 after 3 days of drinking 2% (w/v) [1-13C]-acetate. Palmitate levels were measured in liver whereas cholesterol values were determined in plasma. Values are mean ± SEM (n=6). * p<0.05 vs high fat

Figure 6: Blood glucose levels after a period of nine hours of fasting, after the basal glucose infusion and during the hyperinsulinemic euglycemic clamp. Values are mean ± SEM (n=6). * p<0.05 vs control
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We observed a marked decrease in body weight gain in SP-treated mice compared to high fat-fed mice without indications for intestinal malabsorption or reduced food intake. Peripheral insulin sensitivity was significantly improved in animals treated with Soraphen. De novo lipogenesis in these mice was decreased, indicating that ACC1 was functionally inhibited in these experiments. Alterations of plasma β-hydroxybutyrate levels confirm inhibitory action of Soraphen on ACC2 activity as well.

DISCUSSION

This study demonstrates that pharmacological inhibition of the ACC system provides possibilities for the treatment of diet-induced insulin resistance and obesity. (HF -36±9, SP50 -17±6, SP100 +8±8 % vs control mice). As expected, the metabolic clearance of glucose in HF-fed mice was decreased and back to control levels in both SP-treated groups (Figure 7C). During the clamp, hepatic glucose production (Ra) was reduced by about 50% compared to basal in all groups except in the SP100 group. This group showed a decrease of only 10% (NS vs basal) during the hyperinsulinemic euglycemic clamp (Figure 7D).
Experiments in fungi showed that high doses of Soraphen may have toxic effects [12,13,23]. However, we did not observe any abnormalities with respect to teratogenic effects in both groups treated with Soraphen which was also reflected by basically unchanged ALAT and ASAT values and also by hepatic TNFα gene expression.

Results presented in this paper in part confirm data obtained in the ACC1+/− mice, ACC2−/− mice [1–5,7,24] and rats treated with antisense oligonucleotide (ASO) therapy to block ACC1 and ACC2 [6]. Generalized pharmacological ACC inhibition simultaneously reduced fatty acid synthesis and increased β-oxidation rate. Our data delineate the potential application of pharmacological ACC inhibitors for treatment of key components of the metabolic syndrome. Earlier experiments with CP-640186, another generalized pharmacological ACC inhibitor (own experiments (unpublished results) and from others [8,10,11]), also showed reduced body weight gain on a HF-diet and improved insulin sensitivity upon ACC blockade in mice [8,10,11]. The different approaches clearly establish that inhibition of the ACC system is of potential use for treatment of obesity and insulin resistance.

We observed a feeding behavior similar to that of control and high fat-fed mice in animals treated with Soraphen which was, however, associated with less body weight gain. Fat malabsorption could be excluded as a cause of this effect since fat absorption was similar in HF-fed control mice as in SP100− treated animals. Body fat distribution in SP-treated mice was clearly different from that in HF mice although the caloric intake in these mice was similar. Mice fed the high fat diet supplemented with Soraphen most likely had a higher β-oxidation rate in view of their elevated plasma levels of β-hydroxybutyrate. This has also been reported for ACC2−/− mice fed a high fat diet [4,7]. We observed a clear decrease in the fractional synthesis of palmitate in livers of SP100 mice compared to the HF-fed mice, demonstrating that ACC1 is indeed inhibited by Soraphen treatment. Measurements of energy expenditure are required to elucidate the underlying mechanisms of impaired body weight gain. A remarkable observation was the increase of plasma TG values in mice treated with both doses of Soraphen, whereas in the liver only a tendency towards increased levels was noted. This hepatic effect was caused by the HF diet, since there was no difference between the HF diet alone and the HF diet supplemented with Soraphen. The reason of increased plasma TG levels is still unknown and needs further investigation.

Analysis of gene expression patterns in liver, skeletal muscle and white adipose tissue showed few alterations in response to Soraphen treatment. We only observed an increase in hepatic Fas mRNA expression levels in all three HF-fed groups, likely reflecting Pgc1β-induced response to HF feeding [25,26]. Increased Fas expression, however, was not reflected in increased de novo lipogenesis since Soraphen acts by inhibition of enzyme activity at the protein level. We also observed an increase in hepatic Gk expression levels upon HF feeding. Ferre et al. showed that long-term hepatic overexpression of glucokinase results in a marked insulin resistance in mice
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upon HF feeding [27]. However, other studies on type I and type II diabetes mellitus showed decreased levels of hepatic glucokinase expression [28,29]. The expression pattern of glucokinase in diabetic models apparently strongly depends on the model that is used.

The hyperinsulinemic euglycemic clamp in non-anaesthetized mice was used as the ‘gold standard’ for determining the insulin sensitivity of both liver, related to glucose production, and peripheral tissues, related to the uptake of glucose by several tissues like skeletal muscle, adipose tissue and the brain. Peripheral insulin sensitivity in mice treated with both doses of Soraphen was clearly improved, although no alterations on skeletal muscle or white adipose tissue gene expression patterns were seen. This supports the fact inhibition of ACC by Soraphen indeed improves peripheral insulin sensitivity in mice fed a high fat diet.

Surprisingly, hepatic glucose production (Ra; figure 7D) in mice treated with the highest dose of Soraphen appeared to be insulin resistant. Only a non-significant reduction of Ra by 10% was observed compared to a reduction of 50% in the other three groups during the clamp compared to the basal state. The relatively high dose of Soraphen is most likely the cause of this effect since it was not observed when using either the 50 mg/kg/d dose (Ra; Figure 7D) or CP-640186 in the dose of 50 mg/kg/d (unpublished data). Probably, the therapeutic range of Soraphen is rather narrow. However, in the periphery a clear improvement of insulin sensitivity was observed in the SP100 mice.

From figure 5A it is evident that the acetyl-CoA pool used for de novo lipogenesis is influenced by SP100. The lower precursor-pool enrichment in the presence of Soraphen, compared to control, indicates an increased supply of acetyl-CoA moiety. It is also evident from plasma ketone body concentrations that β-oxidation was stimulated. The question arises whether this increased delivery of lipogenic acetyl-CoA derives from enhanced β-oxidation. Furthermore, induction of β-oxidation might alter pyruvate metabolism. We speculate that an increased carbon flux through pyruvate carboxylase towards oxaloacetate and glucose by gluconeogenesis might result in an apparent insulin resistance of glucose production in the liver. However, no experimental data is available yet to substantiate this suggestion. In this respect, it is of interest to note that Derks et al found only a minor inhibition of gluconeogenesis in vivo in mice during pharmacological inhibition of β-oxidation [30].

In conclusion, our data demonstrate that pharmacological inhibition of the ACC system with Soraphen provides a powerful means to reverse diet-induced obesity and peripheral insulin resistance in mice. In this way, our data provides new options for development of treatment strategies for these components of the metabolic syndrome. We also found, however, that inhibiting hepatic ACC activity might have unexpected consequences for hepatic glucose metabolism. This aspect, pointing to a tight relationship between ACC activity and pyruvate metabolism, requires further attention.
Chapter 4

Acknowledgements

The research of this study was supported by the Dutch Diabetes Research Foundation (grant 2003.00.009).
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