Orlistat treatment of unconjugated hyperbilirubinemia in Crigler-Najjar disease; A randomized controlled trial

CHAPTER 6

Submitted

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ABSTRACT

Unconjugated hyperbilirubinemia in Crigler-Najjar (CN) disease is conventionally treated with phototherapy (PT) and/or phenobarbital (PB). Orlistat treatment decreases plasma unconjugated bilirubin (UCB) concentrations in Gunn rats, the animal model for CN disease. We determined in CN patients the effects of orlistat treatment on plasma UCB concentrations, and on fecal excretion of fat and UCB. A randomized, placebo-controlled, double-blind, cross-over trial was conducted in 16 patients, simultaneous with their regular treatment (PT, n = 11, and/or PB, n = 6). Patients received orlistat or placebo, each for 4-6 weeks (2 weeks interval). Plasma UCB concentrations and fecal excretion of fat and UCB were determined. We defined a clinically relevant response to orlistat treatment as a decrease in plasma UCB concentration of at least 10%. Compared with placebo, orlistat increased fecal fat excretion (+333%, p<0.001), and fecal UCB excretion (+43%, p<0.05). Orlistat treatment decreased plasma UCB concentration by 9% (p<0.01). In 7 of 16 patients, the decrease in plasma UCB levels was clinically relevant (i.e. more than 10%; mean 21%, p<0.001). Only in patients with a clinically relevant response, plasma UCB concentrations during orlistat were strongly, negatively correlated with fecal fatty acid excretion (r = -0.93, p<0.01). Clinically relevant response to orlistat treatment was not correlated with age, sex, CN-type, BMI, or co-treatment with PT or PB, but appeared correlated with a relatively low dietary fat intake of less than 35 energy% (p=0.05). In conclusion, orlistat treatment decreases plasma UCB concentrations, particularly in a subgroup of CN patients. Dietary fat intake may determine the responsiveness to orlistat treatment.
INTRODUCTION

Crigler-Najjar (CN) disease is a genetic disorder of bilirubin metabolism caused by deficiency of the hepatic enzyme bilirubin-UDP-glucuronosyltransferase (UGT1A1). UGT1A1 catalyzes the conjugation of unconjugated bilirubin (UCB), the hydrophobic end product of heme degradation. UCB requires conjugation for its efficient secretion into bile. After biliary secretion, bilirubin is deconjugated in the biliary tree and intestinal lumen, followed by intestinal metabolism, reabsorption and/or fecal excretion. CN patients suffer from permanent unconjugated hyperbilirubinemia which, if left untreated, can cause bilirubin-induced neurologic damage (BIND) and kernicterus, resulting in physical and mental handicaps or even death. The prevalence of CN disease is estimated at 1:1,000,000. In the Netherlands there are approximately 20 patients. Two types of CN disease exist. In type I there is no detectable UGT1A1 activity. Plasma UCB concentrations in untreated type I patients are above 350 µmol/l and can be as high as 800 µmol/l, especially during intercurrent febrile illness. Plasma UCB levels in untreated type II patients are generally below 350 µmol/l because of some (~5%) residual enzyme activity, which can be enhanced by treatment with phenobarbital.

All type I and some type II patients need daily phototherapy to reduce the risk of kernicterus. Phototherapy increases the hydrophilicity and biliary secretion of UCB by photoisomerization. Phototherapy aims to keep plasma UCB levels below 350-400 µmol/l. Life-long daily phototherapy has considerable disadvantages. Main problems are a decreasing efficacy with age and a profound impact of the intensive phototherapy regimen on the quality of (social) life. During exacerbations of jaundice, several measures in addition to continuous high-intensity phototherapy are taken to manage the disease safely, including albumin infusion if the bilirubin-albumin molar ratio is above 0.7, and the avoidance of drugs that displace bilirubin from albumin. Several type I patients have undergone liver transplantation, which restores UGT1A1 activity but has major risks and complications, and requires life-long immunosuppressive treatment. Future treatment for CN disease may be gene therapy. In the animal model for CN disease, the Gunn rat, gene therapy effectively restores UGT1A1 activity. However, vector toxicity and concerns about long-term safety have so far prevented the use of gene therapy in humans.

An alternative treatment option for unconjugated hyperbilirubinemia is based on intestinal capture of UCB by oral treatment. Particularly when plasma UCB concentrations are high as in CN disease, UCB can diffuse from the blood into the intestinal lumen across the mucosa. Additionally, very small amounts of UCB can be excreted into bile. In type II patients the small amounts of conjugates that are formed can be deconjugated to UCB after biliary secretion. Intestinal capture of UCB followed by fecal excretion reduces the enterohepatic circulation of UCB and subsequently decreases plasma UCB concentration. Several orally administered non-absorbable binders of UCB have been applied for intestinal...
capture. Agar,\textsuperscript{22} activated charcoal\textsuperscript{23} and cholestyramine\textsuperscript{24} are no longer used for treatment of unconjugated hyperbilirubinemia because of inconsistent clinical results and side effects.\textsuperscript{25-27} Zinc sulphate was shown to inhibit enterohepatic cycling of UCB, but increased serum zinc levels.\textsuperscript{28} Intestinal capture of UCB by calcium phosphate was very effective in Gunn rats,\textsuperscript{29} but efficacy was less pronounced in patients with CN disease.\textsuperscript{4}

Recently, we demonstrated in Gunn rats that orlistat treatment decreases plasma UCB concentrations parallel with increased fecal fat excretion, and induces net transmucosal excretion of UCB from the blood into the intestinal lumen.\textsuperscript{30-32} In human adults, orlistat has been widely applied for treatment of obesity, without serious side effects.\textsuperscript{33} Recent studies in obese adolescents and prepubertal children indicate that short-term orlistat treatment is well-tolerated by children and generally has only mild side effects.\textsuperscript{34-38} In the present study, we determined the effects of orlistat treatment in patients with CN disease.

**METHODS**

*Study Design.* A randomized, placebo-controlled, double-blind, cross-over clinical trial was conducted in patients with CN disease. The study was performed in the winter season (Sept.-Dec.). All patients started at the same time with the trial to minimize environmental confounding factors, such as variations in sunlight and CYP1A1&2 inducers that catalyze oxidation of UCB. In all patients, current treatment with phototherapy (n = 11) and/or phenobarbital (n = 6) was continued during the trial, *i.e.* orlistat was tested as an adjunct treatment. Patients received orlistat or placebo in a cross-over design with 2 weeks interval, and each patient served as his/her own control. The initial duration of the trial was 12 weeks. The first two weeks was a control period in which the intra-individual variation in plasma bilirubin concentration was determined in three, weekly, samples. Orlistat or placebo would then be taken during 4 weeks, followed by 2 weeks (wash-out) interval and then the alternate treatment during 4 weeks. At the end of the study period, there was a post-treatment control period of 2 weeks. Upon conduction of the trial, however, the dosage of the trial medication had to be adapted after two weeks (see Medication section) and therefore 2 weeks were added to the trial. Total duration of the trial was thus 14 weeks, with treatment periods of 6 and 4 weeks and two control weeks before, in between, and after the treatment periods.

Blood samples were taken weekly to determine plasma UCB concentration. A clinically relevant response to orlistat treatment was defined as a decrease in plasma UCB concentration of at least 10%. This definition was based on the variation in intra-individual plasma UCB concentrations in the control period before start of treatment, which on average was below 10% (mean variation 6 ± 2%). Control UCB values were a mean of 3 weekly samples taken before start of orlistat. Response UCB values were a mean of 2 weekly samples taken around the end of the orlistat treatment period. Samples were taken at approximately the same hour each week to exclude diurnal variation of plasma UCB levels as possible confounding factor,
and to be sure that each week there was an equal time period between nightly phototherapy and blood sampling. Six times during the trial hematological and biochemical parameters were assessed (see Analytical Methods section). In patients treated with phenobarbital, plasma levels were checked every two weeks. Feces were collected 10 times at regular intervals during the trial. Three times 72-hour samples were collected to determine fecal fat excretion; once during each period (control, orlistat, placebo). The other 7 times a small sample of feces was collected. Fecal fat- and UCB concentration was determined in all feces samples.

Patients. Sixteen patients participated in the trial; 13 from The Netherlands, 3 from Belgium. All patients, and/or their parents if the patient was younger than 18 years, gave written informed consent. The study protocol was approved by the Ethics Committees of the Erasmus University Medical Center in Rotterdam, The Netherlands, and of the Hôpital Universitaire des Enfants Reine Fabiola in Brussels, Belgium. To be included in the trial, patients had to be older than 7 years. Exclusion criteria were cholestasis, chronic malabsorption syndrome and pregnancy. Stop criteria were defined as a patient’s wish to stop or serious adverse events, defined as death, life-threatening events, hospitalization or severe side effects (anaphylaxis, an increase of liver transaminases > 30%, an increase of plasma UCB concentration > 50% with risk of kernicterus, a plasma UCB level > 500 µmol/l, severe coagulation problems or diarrhea with > 10% weight loss). During the trial, body weight, blood pressure and pulse rate were recorded weekly and, if indicated, physical examination was performed.

Medication. Orlistat (Xenical®) is a selective inhibitor of gastrointestinal lipases that dose-dependently inhibits hydrolysis of dietary triglycerides. At a recommended dose of 3 times daily (t.i.d.) 120 mg, dietary fat absorption is reduced by approximately 30%. Orlistat and placebo capsules were custom-made by the pharmacist of the Erasmus University Medical Center, Rotterdam. Initially, adults received 120 mg t.i.d. during a meal, and children ~66 mg/m² body surface area t.i.d. (= roughly equal amount as an adult per m² body surface area). However, after two weeks of treatment, some patients suffered from side effects (diarrhea, n = 3) and one patient (patient C, Table 1) from severely increased plasma UCB levels (maximum 451 µmol/l), after which the code was broken in close collaboration with the Ethics Committee. The symptoms were confined to orlistat treated patients, after which the orlistat dosage was reduced by one third. This resulted in the following dosages. Adults: during breakfast and lunch 60 mg, during dinner 120 mg; children: during breakfast and lunch ~33 mg/m² body surface, during dinner ~66 mg/m² body surface area. The study protocol was prolonged by two weeks, to allow patients to be compared for identical durations of treatment with the adapted orlistat dosage (i.e. 4 weeks). The Ethics Committee approved the new study protocol. Cellulose was used as placebo. Previously, cellulose was shown to have no effect on plasma bilirubin concentrations.
Diet. Patients were instructed to eat their normal diet, but were requested not to eat heme-rich food, such as blood sausages or bloody red meat because heme would be converted to bilirubin. Food intake was assessed three times during the trial, once during each period (control, orlistat, placebo). Patients and/or their parents were instructed by a dietician to keep a detailed record of the diet for 3 successive days, at the same days the 72-hour feces samples were collected. Nutrient intake was calculated from a computerized Netherlands food composition database.

Compliance. The importance of medication compliance was stressed regularly during the trial. Compliance was checked by counting remaining capsules afterwards, by determination of fecal fat concentration, and by reviewing the diary patients had been instructed to keep. In this diary, patients had to record daily the times at which the medication was taken, as well as the number and consistency of bowel movements, and side effects, if any. Plasma levels of phenobarbital were determined every other week. With regard to phototherapy compliance, patients and/or their parents were instructed to use the lights for approximately the same amount of hours every day and to keep a detailed record of phototherapy times. Furthermore, light levels were checked weekly with a Lux meter. Since in 14 weeks light levels would deteriorate too much if the same light bulbs were used, half of the light bulbs were replaced by new bulbs two weeks before start of the trial; the other half of the bulbs were replaced halfway the trial in the wash-out period.

Analytical Methods

Blood. Directly after vein puncture, tubes were protected from light and kept cool until analysis. All analytical procedures were performed in dim light with routine clinical chemical procedures. For bilirubin measurements, blood samples were processed immediately. Plasma bilirubin levels were determined twice per sample. First, plasma total bilirubin level was determined on a Hitachi 912 analyzer (Roche, Mannheim, Germany). The remaining plasma was kept in the dark at -80ºC under nitric oxide gas, until analysis of plasma UCB concentration by reversed-phase HPLC, as described previously. Hemoglobin (Hb), hematocrit (Ht) and reticulocytes were determined on a Advia 120 hematology analyzer (Bayer, Leverkusen, Germany). Aspartate-aminotransferase activity (AST) and albumin were determined on a Hitachi 912 analyzer, activated partial thromboplastin time (APTT) and protrombin time (PT) on a Sysmex CA-1500 analyzer (Sysmex UK, Milton Keynes, UK), and phenobarbital levels on a DDX-FLX analyzer (Abbott Diagnostics, Hoofddorp, The Netherlands). Vitamin E levels were determined by HPLC.

Feces. Feces were stored in the dark at -20ºC until analysis. For determination of fatty acids in feces, aliquots of homogenized feces were extracted, hydrolyzed and methylated according to the method of Lepage and Roy, with the modification that methanol/hexane (4:1, v/v) was used for methylation and extraction. Resulting fatty acid methyl esters were determined by
gas chromatography (HP Ultra 1 column, Hewlett-Packard, Palo Alto, CA), and fatty acid contents were calculated in molar amounts, using C17:0 as internal standard. Fecal fat excretion was also determined via Near Infra Red Assay (NIRA) of homogenized 72-hour feces samples using a modification of the Van de Kamer method.\textsuperscript{41} Total fat analysis was performed by using the Soxtec® Avanti 2050 system (FOSS Tecator AB, Hoganas, Sweden), which utilizes a new, patented four-step solvent (hexane) extraction technique. The entire process is fully automated, including hydrolysis, filtration, washing and drying. Before performing the automated extraction method, 4 gram of feces was dried overnight at 103°C. Fecal fat concentration was expressed as gram per 24-hour collection period. For determination of UCB in feces, aliquots of homogenized feces were submitted to alkaline methanolysis and chloroform extraction. After evaporation under nitrogen, the residue was re-dissolved in chloroform and analyzed by reversed-phase HPLC, as described previously.\textsuperscript{32}

**Statistical Analyses**

Power analysis was performed before start of the trial.\textsuperscript{42,43} Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD. Based on a normal distribution of plasma UCB levels in CN patients, parametric tests were used for statistical analysis. Intra-individual differences were tested with the paired Student $t$ test. The relationship between plasma UCB and fecal fat was analyzed by linear regression analysis. Correlation analysis was done with Pearson’s cross-tables. $P$ values less than 0.05 (two-tailed) were considered significant.

**RESULTS**

**Patient characteristics**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>CN Type</th>
<th>Age (years)</th>
<th>Sex (M/F)</th>
<th>Body Weight (kg)</th>
<th>BMI (kg/m$^2$)</th>
<th>UCB Concentration (mg/day)</th>
<th>Phototherapy (hours/day)</th>
<th>No. bulbs / Watt</th>
<th>Phenobarbital (mg/day)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>I</td>
<td>10</td>
<td>F</td>
<td>41</td>
<td>20</td>
<td>10 ± 11</td>
<td>20 ± 10</td>
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<td></td>
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<tr>
<td>B</td>
<td>I</td>
<td>11</td>
<td>F</td>
<td>37</td>
<td>18</td>
<td>8 ± 10</td>
<td>12 ± 10</td>
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<td></td>
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<td>C</td>
<td>I</td>
<td>14</td>
<td>M</td>
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<td>10 ± 10</td>
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<td>I</td>
<td>14</td>
<td>F</td>
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<td>M</td>
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<td>F</td>
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<td>10 ± 150</td>
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<td>F</td>
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<td>23</td>
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<td>Phenobarbital 1 x 200*</td>
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<td></td>
</tr>
<tr>
<td>L*</td>
<td>II</td>
<td>28</td>
<td>F</td>
<td>85</td>
<td>25</td>
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<td>1 ± 75, 2 ± 50</td>
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<td></td>
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<td>M</td>
<td>II</td>
<td>30</td>
<td>F</td>
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<td>10 ± 150</td>
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<td>F</td>
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<td>P</td>
<td>II</td>
<td>51</td>
<td>M</td>
<td>74</td>
<td>23</td>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
</tbody>
</table>

BM: Body Mass Index, calculated as: body weight (kg)/height (m$^2$). CN: Crigler-Najjar

*Phenytoine instead of phenobarbital. *Patient quit during the trial after 10 weeks.
Sixteen patients with Crigler-Najjar disease participated in the trial (Table 1). Seven patients had type I CN disease and 9 patients type II. Half of the patients were below the age of 18 years. Median age was 17.5 years (range 8-51 years). Male to female ratio was 5:11 (31%:69%). Median body weight was 64 kg (range 29-85 kg). Median BMI was 22.5 (range 16-30). Phototherapy treatment was necessary for 11 patients. The amount of hours of daily phototherapy ranged from an average of 9.4 hours in type I patients to 1 or 2 hours in some type II patients. Plasma UCB concentrations of one type II patient were in the high range of type I patients, but on the basis of a response to phenobarbital treatment this patient was diagnosed with type II CN disease. Five type II patients were treated with phenobarbital and one with phenytoïne.

<table>
<thead>
<tr>
<th>Table 2. Hematological and biochemical parameters</th>
<th>control</th>
<th>orlistat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mmol/l)</td>
<td>8.8 ± 0.6</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>Hematocrit (v/v)</td>
<td>0.41 ± 0.03</td>
<td>0.40 ± 0.03</td>
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<tr>
<td>Reticulocytes (%)</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>67 ± 69</td>
<td>63 ± 47</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>45 ± 2</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>33 ± 4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Vitamin E (mmol/l)</td>
<td>22 ± 5</td>
<td>18 ± 4*</td>
</tr>
</tbody>
</table>

*p<0.001

**General effects of orlistat**

Orlistat treatment did not significantly affect body weight (average body weight without orlistat: 64 ± 16 kg vs orlistat: 64 ± 17 kg, NS). Table 2 shows the effects of orlistat on hematological and biochemical parameters. Hemoglobin, hematocrit and reticulocyte count were not affected by orlistat treatment, in accordance with absence of relevant hemolysis and with stable bilirubin production during the trial. Plasma albumin concentrations, ALT, APTT, and PT levels were not affected by treatment. Orlistat treatment significantly decreased vitamin E levels (-18%, p<0.001). In patients treated with phenobarbital, plasma levels were stable during the experimental period, based on 2-weekly sampling (average levels without orlistat: 13.0 ± 3.5 mg/l, vs orlistat: 12.5 ± 4.0 mg/l, NS).

**Effects of orlistat on plasma UCB concentrations**

Figure 1 shows the effects of orlistat treatment on plasma UCB concentrations in Crigler-Najjar patients. Before orlistat treatment, plasma UCB levels ranged from 123 to 354 µmol/l among the patients (Figure 1A). Average plasma UCB concentrations per group were 317 ± 36 µmol/l for type I patients and 228 ± 71 µmol/l for type II patients. Within each patient, plasma UCB levels were fairly constant (mean variation 6 ± 2%). Plasma UCB levels measured by HPLC were used for calculations. Levels measured on the Hitachi analyzer were similar (mean difference <5%). Orlistat treatment decreased plasma UCB concentrations by 9
± 12% (p<0.01; range -32 to +8%; Figure 1B). Based on the definition of a clinically relevant response to treatment, 7 of the 16 patients (44%) responded, with a mean decrease of 21% in plasma UCB levels (range 11 to 32%).

**Figure 1.** Effects of orlistat treatment on plasma UCB concentrations in Crigler-Najjar patients. Patients were treated for 4 weeks with orlistat. Plasma UCB concentration was measured weekly. Control UCB values were a mean of 3 weekly samples taken before start of orlistat. Orlistat UCB values were a mean of 2 weekly samples taken around the end of the orlistat treatment period. Each set of triangles represents data from one patient. Response to orlistat treatment was defined as a decrease in plasma UCB concentration of at least 10%. ▲ responders, △ non-responders.

**Effects of orlistat on fecal fat- and UCB excretion**

Orlistat treatment profoundly increased fecal fat concentration in all patients (+333 ± 249% according to gas chromatographic analysis of fecal fatty acids; p<0.001; data not shown), and total fecal fat excretion (+652 ± 516% according to the NIRA method of fecal fat analysis; p<0.001; Figure 2). Fecal excretion expressed per kilogram body weight increased from 0.05 ± 0.02 to 0.28 ± 0.12 g/kg/24h (+652 ± 516%, p<0.001). Fecal fat concentration and excretion were not different between orlistat responsive and non-responsive patients. Orlistat treatment also increased fecal concentration of UCB (+43%, p<0.05; range -46 to +223%; Figure 3). Under control conditions, fecal UCB concentrations varied between patients from 0.05 to 0.31 µmol/gram feces (mean 0.14 ± 0.08). Fecal UCB concentrations did not differ significantly between orlistat-responsive and non-responsive patients, either under control conditions or during orlistat treatment.

**Figure 2.** Effects of orlistat treatment on fecal fat excretion in Crigler-Najjar patients. Patients were treated for 4 weeks with orlistat. 72-hour fecal fat excretion was measured 3 times. Control values were a mean of two 72-hour samples taken during control and placebo periods. Orlistat values were single 72-hour samples taken during orlistat treatment. Each set of triangles represents data from one patient. Response to orlistat treatment was defined as a decrease in plasma UCB concentration of at least 10%. ▲ responders, △ non-responders.
In previous studies in Gunn rats, the change in plasma UCB concentration was strongly correlated with the amount of fat excreted via the feces.\textsuperscript{30-32} Figure 4 shows the relationship between parameters of fecal fat excretion and the orlistat-induced alteration in plasma UCB concentration. For all patients combined, the UCB response to orlistat was only minimally related to the relative stimulation of fecal fat excretion or to the absolute amount of fecal fat excretion (Figure 4; trend lines not shown). Upon separate analysis of responding and non-responding CN patients, however, marked differences were observed. Plasma UCB concentrations in responding CN patients were strongly, negatively related to parameters of fecal fat excretion ($r = -0.93$, $p<0.01$, Figure 4). In contrast, plasma UCB concentrations in non-responding patients were independent of fecal fat excretion. Fat absorption (calculated as the difference between total fat intake (g/24h) and fecal fat excretion (g/24h) appeared to be less in the responding patients compared with the non-responding patients (58 ± 31 and 86 ± 31 g/24h, respectively, $p=0.11$).

\textbf{Figure 3.} Effects of orlistat treatment on fecal UCB excretion in Crigler-Najjar patients. Patients were treated for 4 weeks with orlistat. Fecal UCB excretion was measured regularly. Control values were a mean of 5-6 samples taken during control and placebo periods. Orlistat values were a mean of 4-5 samples taken during orlistat treatment. Each set of triangles represents data from one patient. Response to orlistat treatment was defined as a decrease in plasma UCB concentration of at least 10%. ▲ responders, △ non-responders.

\textbf{Figure 4.} Negative linear relationship between plasma UCB concentration during orlistat treatment (compared with pre-treatment values) and fecal fat excretion during orlistat in responding Crigler-Najjar patients. Patients were treated for 4 weeks with orlistat. 72-hour fecal fat excretion was measured. Each triangle represents data from one patient. Response to orlistat treatment was defined as a decrease in plasma UCB concentration of at least 10%. ▲ responders, △ non-responders. One responder did not collect 72-hour feces.
Characteristics of responsive vs. non-responsive patients

Body weights of the patients varied between 29 and 85 kg (Table I). The observed differences between responding and non-responding patients were similar upon expression of fecal fat excretion relative to body weight. Also, the responders and non-responders did not significantly differ in the amount of saturated or unsaturated fatty acids excreted via the feces, nor in their relative (molar) ratio in feces (Figure 5). Accordingly, neither saturated nor unsaturated fecal fat appeared specifically related to plasma bilirubin concentrations.

![Figure 5](distribution-un saturated-saturated-fatty-acids-in-feces-of-crigler-najjar-patients-during-orlistat-treatment.jpg)

In a study in which Crigler-Najjar patients were treated with calcium phosphate, differences in response to treatment correlated with the type of CN disease (type I or II) and with co-treatment with phototherapy. Since our data also indicated differences in responsiveness to orlistat treatment, we analyzed whether these or other patient characteristics correlated with (non-)responsiveness (Table 3). The (non-)responsiveness of plasma UCB concentrations to orlistat treatment did not significantly correlate with either CN type, age, sex, body weight, or co-treatment with phototherapy or phenobarbital. Interestingly, however, the majority of the responsive patients had a total dietary fat intake below 35 energy% (p=0.05), and all responsive patients had a BMI equal to or below 25 (p=0.10).

| Table 3. Correlations (non)-respons vs patient characteristics / dietary intake |
|-----------------------------------------------|-----------------|-----------------|-----------|
| Patient characteristics                      | Non-responders  | Responders      | P value   |
| CN type                                      | 44%             | 43%             | 0.95      |
| Age                                          | <18 years       | 44%             | 0.64      |
| Sex                                          | Female          | 56%             | 0.22      |
| BMI                                          | ≤25             | 67%             | 0.10      |
| Phototherapy                                 | Yes             | 78%             | 0.41      |
| Phenobarbital*                               | Yes             | 33%             | 0.72      |
| Dietary intake < average per day             | Non-responders  | Responders      | P value   |
| Energy                                       | <2200 Cal       | 56%             | 0.95      |
| Total fat                                    | <35 energy%     | 22%             | 0.05      |
| Saturated fat                                | <13 energy%     | 33%             | 0.72      |
| Cholesterol                                  | <220 mg         | 33%             | 0.15      |
| Calcium                                      | <800 mg         | 67%             | 0.85      |
| Fiber                                        | <17 g           | 67%             | 0.42      |

*In one patient Phenytoine in stead of Phenobarbital
Dietary intake of saturated fat, cholesterol, calcium or fiber was not significantly different between orlistat responsive and non-responsive patients.

Side effects & compliance

As mentioned in the Methods section, after two weeks of treatment the dosage of orlistat had to be adapted because of side effects. Side effects were almost exclusively related to the gastrointestinal tract, such as diarrhea, oily leakage, stomach cramps, flatulence and fecal urgency. After dosage reduction the side effects decreased in severity and for the rest of the study they were generally mild, temporary, and tolerable for all patients. Oily leakage and diarrhea were remarkably yellow/orange colored. Mild side effects such as greasy appearance of the feces occurred in almost all patients. Some side effects (such as flatulence) were also reported during placebo treatment. Systemic side effects were not observed. Headache was incidentally reported, equally during placebo and orlistat treatment. Five patients reported increased energy levels and well-being during orlistat treatment. Three patients reported increased appetite during orlistat. One patient (patient C, table 1) stopped during one week with orlistat treatment because of an increase in plasma bilirubin levels after one week of treatment. After dosage reduction the treatment was restarted and one week was added to the treatment period of this patient. Another patient (patient L, table 1) dropped out from the trial at ten weeks, because of psychological issues (a.o. lack of concentration, mood swings). Compliance to medication appeared to be good in all patients, based on capsule counting, corresponding diary entries, and, by inference, from increased fecal fat excretion during orlistat treatment. With regard to phototherapy, patients spent the same number of hours under the phototherapy lights during control periods and placebo or orlistat treatment. Light levels checked weekly with a Lux meter, showed that lamps deteriorated during the study period by approximately 10%.

DISCUSSION

We determined the effects of orlistat treatment on plasma unconjugated bilirubin concentrations in patients with Crigler-Najjar disease. In general, orlistat treatment slightly decreased plasma UCB concentrations. Although this was a statistically significant decrease, it was not considered clinically relevant for the whole group, based on our definition of treatment response. Interestingly, however, the effect was more pronounced in a subgroup of patients. In this orlistat responsive subgroup, the decrease in plasma UCB concentration was strongly related to orlistat-induced fecal fat excretion. The responsiveness of CN patients could not be predicted by pre-treatment patient characteristics, except that the responsive patients tended to have a lower dietary fat intake and lower BMI. Our definition of treatment response (a decrease in plasma UCB concentration of at least 10%) was based on the variation in intra-individual plasma UCB concentrations before start of treatment, which was on
average below 10%. Our cutoff level of 35% energy% for separating a “high” versus “low” dietary fat intake was based on our dietician’s guideline for a “healthy” diet that recommends a dietary fat intake between 30-35 energy%.

Previously, we demonstrated in Gunn rats that orlistat treatment decreased plasma UCB concentrations by 20-40%, \(^{30,32}\) i.e. more pronounced than presently observed in CN patients. A species difference or the length of the intestine could possibly explain the different efficacies of orlistat treatment. Rats have a relatively much larger surface area of intestinal mucosa than humans and therefore transmucosal excretion of UCB during orlistat treatment might be more pronounced in rats than humans. Other potential reasons include type of diet and differences in intestinal microflora. Our observations are similar to those obtained with calcium phosphate treatment by Van der Veere \textit{et al.} Calcium phosphate strongly decreased plasma bilirubin levels in Gunn rats, but the efficacy was limited in CN patients.\(^ {4,29}\) Also, calcium phosphate was only effective in a subgroup of patients, namely in patients receiving phototherapy (mostly type I patients).

The responsiveness to orlistat treatment was independent of co-treatment with phototherapy or phenobarbital, CN-type, body weight, sex, or age. However, the subgroup of patients that responded to orlistat treatment did appear to have a lower dietary fat intake and lower BMI than non-responsive patients. Previously, we showed in Gunn rats that orlistat treatment was more effective on a low-fat than a high-fat diet.\(^ {30}\) Dietary fat intake thus appears to be a relevant factor in determining efficacy of orlistat treatment. Possibly, patients with a high fat intake (and consequently higher fecal fat excretion) have already reached a maximum level of UCB capture by fat and increasing fat intake and/or excretion may therefore not (further) decrease plasma UCB concentrations. Present data clearly identify the subgroup in one specific phenomenon: only in the CN patients with a clinically relevant response to orlistat treatment, plasma UCB levels were strongly, negatively correlated with fecal fat excretion. Previously, we showed a similar relationship upon orlistat treatment in Gunn rats.\(^ {32}\) The mechanism underlying the presence or absence of this relationship in specific patients remains partly unclear. We speculate that, in addition to dietary fat intake, intestinal factors such as composition or metabolic activity of the intestinal microflora play an important role. Previously, we showed in Gunn rats that the hypobilirubinemic effect of orlistat is partially the result of increased metabolism of UCB to derivatives.\(^ {31}\) Others have shown the importance of the intestinal microflora for UCB metabolism.\(^ {44}\) Gastrointestinal lipase activity levels of the patient may also play a role. The dose of orlistat should perhaps be individualized to generate a certain degree of fat malabsorption with a certain distribution of fatty acids or partially hydrolyzed triglycerides in the intestinal lumen to create an optimal environment for capture of UCB.

The number of CN patients that could participate in the trial was inevitably limited due to the low prevalence of the disease. To overcome this, a cross-over study design was chosen. We obtained several indications that both the duration of the treatment and of the wash-out
period may have been too short, and may have resulted in underestimation of the orlistat effect. Firstly, the majority of patients appeared not to have reached a steady-state in bilirubin homeostasis during the treatment, based on the increased fecal bilirubin content at the end of orlistat treatment. Secondly, in the first week of the wash-out period, plasma UCB concentrations did not readily return to pretreatment values. We cannot exclude the possibility that the hypobilirubinemic effects of orlistat are more pronounced upon prolonged treatment.

Orlistat treatment, at the (eventual) dose used, did not cause major side effects in the CN patients. The generally mild, temporary and tolerable side effects that did occur were similar to those reported in trials where orlistat was used for treatment of obesity. Side effects were almost exclusively related to the gastrointestinal tract. Orlistat treatment decreased plasma vitamin E concentrations. The fat-soluble vitamin E was shown by others to decrease when orlistat was used to treat obesity. Low plasma vitamin E levels (or other fat-soluble vitamins) due to prolonged orlistat treatment could easily be prevented by dietary supplementation.

In conclusion, present data indicate that orlistat can be useful for treatment of unconjugated hyperbilirubinemia, particularly so in a subgroup of CN patients. Dietary fat intake may determine the responsiveness to orlistat treatment. It will be a challenge for future research to identify other relevant parameters or patient characteristics which could predict the responsiveness of CN patients to orlistat treatment, or indeed other patients with unconjugated hyperbilirubinemia. In addition, it needs to be addressed whether prolonged orlistat treatment could safely and reliably decrease the need for phototherapy.
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