Effects of simmondsin on food intake, growth, and metabolic variables in lean (+/? ) and obese (fa/fa) Zucker rats


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Incorporation of 2.5 g/kg of the anorexigen, simmondsin, in the diet resulted in food intake reduction in both lean and obese Zucker rats; however, the obese rats were much more sensitive to the food intake-reducing activity of simmondsin. In both obese and lean simmondsin-treated Zucker rats, growth was slower than in control rats, but was the same as that in pair-fed animals. The 24 h heat production pattern showed a smaller diurnal variation and a lower mean in obese rats than in lean rats. Food intake reduction, as a result of either simmondsin treatment or pair feeding, caused a decrease in mean heat production. Simmondsin treatment, but not pair feeding, caused a decrease in the diurnal variation of heat production. Plasma total cholesterol levels were increased in both simmondsin-treated and pair-fed obese and lean Zucker rats compared with control animals; this increase was mainly due to an increase in HDL-cholesterol levels. Blood leptin levels in both obese and lean rats decreased with decreased food intake and decreased fat deposition, but in obese rats, simmondsin treatment resulted in an additional decrease in leptin levels. It is concluded that the food intake-reducing effect of simmondsin is more pronounced in obese Zucker rats than in their lean littermates, and except for the simmondsin-specific effects on leptin and total cholesterol values in obese littermates, the effects of simmondsin are related to food intake restriction in obese and lean Zucker rats.

Simmondsin: Food intake: Heat production

Simmondsin is a cyano-methylene-cyclohexyl glycoside (Elliger et al. 1973) that occurs in the seeds of the jojoba plant (Simmondsia chinensis), an evergreen shrub that grows in arid and semi-arid environments. Simmondsin is known to induce food intake reduction and weight loss when administered orally in rats (Booth et al. 1974). The food intake reduction caused by simmondsin in rats is persistent and dose-dependent (Cokelaere et al. 1996). Since its effect is much more pronounced in non-fasted rats than in fasted rats, it was postulated that simmondsin causes food intake reduction by inducing satiety and not aversion (Cokelaere et al. 1995a; G Flo, M Van Boven, S Vermaut, P Daenens, E Decuypere and M Cokelaere, unpublished results). The physiological effects of chronic simmondsin administration are identical to those seen with chronic cholecystokinin (CCK) administration (Flo et al. 1998), and administration of devazepide, a potent peripheral CCK receptor blocker, abolishes the food intake-reducing activity of simmondsin (Cokelaere et al. 1995b). Moreover, the anorexic effect of simmondsin is, at least partly, vagally mediated (G Flo, M Van Boven, S Vermaut, P Daenens, E Decuypere and M Cokelaere, unpublished results). On the basis of these observations, it was postulated that simmondsin acts by stimulation of the endogenous CCK system.

Obese Zucker rats are reported to be less sensitive than their lean littermates to the satiating action of exogenously administered CCK (McLaughlin & Baile, 1980; McLaughlin et al. 1982a, b). However, stimulation of endogenous CCK production (by trypsin inhibitors or phenylalanine administration) results in a more marked effect on food intake in obese Zucker rats than in lean Zucker rats (McLaughlin et al. 1982, 1983a, b).

Abbreviations: BW, body weight; C, control; CCK, cholecystokinin; GEI, gross energy intake; HP, heat production; PF, pair-fed; S, simmondsin-treated.

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Obese Zucker (fa/fa) rats are known to have an inefficient long-form leptin receptor, leptin being the product of the obese gene, and to develop hyperphagia and obesity from an early age (Takaya et al. 1996). Obese Zucker rats have higher circulating leptin levels, which are not reduced after 24 h fasting, whereas, in lean Zucker rats, leptin levels are reduced after fasting (Hardy et al. 1996). Energy retention in obese Zucker rats is reported to be more efficient than in lean Zucker rats (Bach et al. 1981). Obese rats show increased adipose tissue deposition from an early age, have increased plasma cholesterol and insulin levels and, when older, also have increased blood glucose levels (Bach et al. 1981). Obese Zucker rats have a lower mean locomotory activity and a lower mean body temperature than their lean litter mates (Fukagawa et al. 1988; Murakami et al. 1995). It is not known whether autonomous food intake reduction makes these values for activity, energy retention efficiency, adipose tissue deposition and plasma leptin and cholesterol levels more similar to those seen in lean Zucker rats.

The aims of the present study were: (1) to examine whether the anorexic effect of simmondsin is more pronounced in obese Zucker rats than in lean Zucker rats; (2) to compare the effect of simmondsin treatment on metabolic variables in obese and lean Zucker rats by measuring heat production, fat deposition, and blood cholesterol, HDL and leptin levels in obese and lean simmondsin-treated and control Zucker rats.

Materials and methods

Animals and housing

Twenty-four lean (+/-fa or +/-t) and twenty-four obese (fa/ fa) 6-week-old male Zucker rats, purchased from Iffa Credo (L’Arbresle, France), were housed two to a cage under standard laboratory conditions (22 ± 2°C, 40–60% relative humidity, lights on from 08.00 to 20.00 hours). Water was provided ad libitum, and complete powdered rodent food (10.1 kJ metabolizable energy/kg, 180 g crude protein/kg, Carfil, Belgium) was provided in special feeders designed to avoid spilling (Scholtz, Overijse, Belgium).

General experimental design

After a 10 d adaptation period, the twenty-four lean Zucker rats (180 g (SE 2.0) g) were divided into three groups of eight rats, two rats per cage. One group had free access to normal rat chow, and served as the control (C) group (group C/lean). A second group received 2.5 g simmondsin/kg (S) mixed in the normal rat chow offered ad libitum (group S/lean). The third group was pair-fed (PF) to the S/lean group, receiving the same amount of food at 17.00 hours (weeks 1, 2 and 4) or 12.00 hours (week 3) as that eaten by the S/obese rats the previous day (group PF/lean).

In a similar way, the twenty-four obese rats (199 g (SE 3.3) g) were divided into (1) a C/obese group having free access to normal rat chow, serving as control group; (2) an S/obese group, which received 2.5 g simmondsin/kg mixed in the normal rat chow offered ad libitum, and (3) a PF/obese group, which was pair-fed to the S/obese group, receiving the same amount of food at 17.00 hours (weeks 1, 2 and 4) or 12.00 hours (week 3) as that eaten by the S/obese rats the previous day. Daily food intake was recorded and body weight (BW) checked three times weekly. Since this experiment was carried out on growing animals, the absolute food intake values were corrected for metabolic BW, food intake being expressed as g/kg BW0.75. The food intake of the S/lean and PF/lean rats was expressed as a percentage of the food intake of C/lean rats, and the food intake of the S/obese and PF/obese rats as a percentage of the food intake of C/obese rats.

After 4 weeks, rats were anaesthetized with diethyl ether between 08.00 and 10.00 hours, then blood was taken by cardiac puncture and the plasma separated by centrifugation and stored at −20°C until used to measure leptin, total cholesterol and HDL levels. The epididymal and perirenal fat pads were dissected immediately, and weighed.

Heat production

Heat production (HP) was measured during the third week of the experiment. To study differences in the HP of simmondsin-treated, pair-fed and control obese and lean rats, groups of three rats from each of the six treatment groups (C/lean, S/lean, PF/lean, C/obese, S/obese, PF/obese) were placed in six environmentally-controlled respiration units (300 × 500 × 560 mm). Between 11.00 and 12.00 hours daily, the respiration chambers were cleaned, food intake measured, pair feeding performed, the rats weighed and the groups randomly assigned to a respiration chamber. O2 consumption and CO2 output were recorded on three consecutive days by paramagnetic and infrared techniques respectively (Gas Handling Unit; Analytical Company Ltd, Hoddesdon, Herts., UK). This same experimental design was used during another period of three consecutive days, using a different three rats from each treatment group.

HP was calculated using the formula according to Westerterp (1994): (HP (kJ/h) = 16.2 × O2 (litres/h) + 5.4 × CO2 (litres/h)) and expressed per kg BW0.75 and per day (Buyse et al. 1992).

Products and plasma analyses

The simmondsin used was extracted and purified as described previously (Van Boven et al. 1993) and was pure on HPLC.

Leptin assay. Blood leptin levels were measured using a commercially available radioimmunoassay kit (Mediagnost, Tübingen, Germany).

Cholesterol and HDL assay. Cholesterol and HDL levels were determined by a clinical assay procedure (Synchron CX system Multi; Beckman Instruments, Brea, CA, USA). All samples were analysed simultaneously, the intra-assay variability being 3-0%.

Statistical analysis

All results are expressed as means with their standard errors (SEM). Statistical analysis was performed by ANOVA (Instat v. 2.04a, © 1990 Graphpad Inc., Sorrento Valley, San Diego, CA, USA) followed by a Student–Newman–Keuls multiple comparison test for significance, with P < 0.05.
being considered a significant difference. Using the approach of Halberg et al. (1972), the theoretical HP curve, \( y = A_0 + A \cos (\omega (t + \phi)) \), was fitted, using cosinor analysis, where \( y \) is the theoretical HP value (kJ/h per kg BW\(^{0.75}\)), \( A_0 \) the mean HP value, \( A \) the amplitude (the maximal deviation from \( A_0 \)), \( \phi \) the acrophase (time at which the maximal positive deviation from \( A_0 \) occurs), \( \omega \) the angular frequency (one cycle/24 h) and \( t \) the time.

**Results**

**Food intake and weight change**

At the start of the experiment, the obese Zucker animals (199.3 (SE 3.3) g) were heavier than the lean animals (180.0 (SE 2.0) g, \( P < 0.0001 \)). Over the 4-week experimental period, obese control rats had an average food intake of 64.6 (SE 2.1) g/kg BW\(^{0.75}\) daily, while that of the lean control animals was 54.9 (SE 1.4) g/kg BW\(^{0.75}\). In both lean and obese simmondsin-treated rats, food intake was significantly reduced compared with their respective ad libitum-fed controls (Fig. 1). The proportional food intake reduction in S/obese rats was higher than in S/lean rats, the difference being statistically significant from the second week onwards (\( P < 0.01 \)) (Fig. 2), while the food intakes of S/obese and S/lean rats were similar from the second week onwards (Fig. 1). Due to the lower food intake, simmondsin-treated rats and their pair-fed counterparts gained weight at a slower rate than the control animals. Similar weight changes were seen in S/obese and PF/obese rats and in S/lean and PF/lean animals (Fig. 3).

**Heat production**

Daily HP patterns are shown in Fig. 4 and the corresponding cosinor fitting data are shown in Table 1. For all treatments, HP showed a significant diurnal pattern (\( A > 0, P < 0.001 \)), but obese rats displayed a significantly smaller amplitude (\( A \)) and a lower mean HP value (\( A_0 \)) than lean rats. ANOVA showed that the difference in mean HP was related to a lower hourly HP during the dark period in the obese rats compared with the lean rats, whereas the mean HP during the light period was the same in lean and obese rats (results not shown). Simmondsin treatment and pair feeding significantly reduced \( A_0 \) in both obese and lean rats. The amplitude of the HP was significantly reduced by simmondsin treatment in both lean and obese rats, but not by pair feeding. In contrast, in PF/obese rats the amplitude was increased compared with C/obese rats. The HP of simmondsin-treated rats (both obese and lean) was maximal during the dark period (Table 1, acrophase), whereas, for the PF/obese and PF/lean rats, the maximal HP occurred during the light period. In all groups, the obese animals tended to reach their maximal HP somewhat earlier than the lean animals (Table 1).

Table 2 shows that, although C/obese rats had a higher gross energy intake (GEI), they lost less energy through HP than did C/lean rats. The HP:GEI value was significantly lower in C/obese rats than in C/lean rats. In both lean and obese rats, the GEI and the HP were significantly reduced by either simmondsin treatment or pair feeding. However, in S/lean and PF/lean rats, the HP:GEI value did not differ from that in C/lean rats, whereas in obese rats the food intake...
reduction caused by simmondsin treatment or pair feeding significantly increased the HP:GEI value.

**Fat deposition**

In both lean and obese Zucker rats, white adipose tissue deposits were smaller in simmondsin-treated and in pair-fed rats than in control animals (Table 3). The weights of the perirenal and epididymal fat pads were the same in S/obese and PF/obese rats. In lean Zucker rats, the epididymal fat pad weighed less in S/lean rats than in PF/lean animals.

**Plasma levels of cholesterol and HDL**

Following simmondsin treatment, plasma levels of cholesterol and HDL increased in both lean and obese rats (S/lean and S/obese rats).
S/obese; Table 4). HDL and total cholesterol levels in the pair-fed animals also tended to increase compared with controls, but the difference was not statistically significant.

Plasma leptin levels

Plasma leptin levels in obese Zucker rats were higher than in lean Zucker rats (Table 3). In lean animals, leptin levels in S/lean and PF/lean animals were identical, but
significantly lower than in C/lean rats. Plasma leptin levels in PF/obese and S/obese rats were significantly lower than in C/obese rats, but S/obese rats showed an additional decrease in plasma leptin levels compared with PF/obese rats.

Discussion

The present results confirm the earlier observations that simmondsin reduces food intake in a persistent way and that this can account for the growth reduction seen in simmondsin-treated rats, since the growth curves of simmondsin-treated and pair-fed rats are similar (Cokelaere et al. 1992, 1996). Although lean Zucker rats showed a proportional food intake reduction that could be predicted from earlier observations in Wistar rats (Cokelaere et al. 1996), obese Zucker rats showed a greater proportional food intake reduction than expected from these earlier results. Moreover, simmondsin treatment also abolished the relative hyperphagia seen in obese rats compared with lean rats.

Table 3. Body weight, weights of perirenal and epididymal white adipose tissue depots and plasma leptin concentration in control (C), simmondsin-treated (S) and pair-fed (PF) obese and lean Zucker rats†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Plasma leptin (ng/ml)</th>
<th>White adipose tissue weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>C/obese</td>
<td>329</td>
<td>3.8</td>
<td>55.7</td>
</tr>
<tr>
<td>S/obese</td>
<td>244</td>
<td>7.7</td>
<td>35.8</td>
</tr>
<tr>
<td>PF/obese</td>
<td>234</td>
<td>5.6</td>
<td>40.6</td>
</tr>
<tr>
<td>C/lean</td>
<td>273</td>
<td>4.1</td>
<td>3.66</td>
</tr>
<tr>
<td>S/lean</td>
<td>219</td>
<td>5.9</td>
<td>2.01</td>
</tr>
<tr>
<td>PF/lean</td>
<td>220</td>
<td>3.5</td>
<td>2.17</td>
</tr>
</tbody>
</table>

a,b,c Mean values within a phenotype not sharing a common superscript letter were significantly different, P < 0.05. Mean values were significantly different from those for the corresponding obese groups, *P < 0.05.
† For details of diets and procedures, see p. 160.
The reason for this more pronounced food intake reduction in obese rats remains to be investigated in depth, but several mechanisms may be proposed. One possible mechanism involves the effect of simmondsin on the CCK system. The fact that simmondsin treatment interferes with the endogenous CCK system is well documented (Cokelaere et al. 1995a,b; Flo et al. 1998). Both CCK and simmondsin induce food intake reduction, at least partially, through stimulation of the vagus nerve (Joyner et al. 1993; G Flo, M Van Boven, S Vermaut, P Daenens, E Decuyper and M Cokelaere, unpublished results). Since obese Zucker rats are more sensitive to the effects of endogenous CCK on food intake than lean rats (McLaughlin et al. 1982, 1983a,b), it is probable that obese Zucker rats are also more sensitive to the satiating effects of simmondsin. Second, the differences in feeding pattern between obese and lean rats must be considered. Since obese rats are deficient in the long-form leptin receptor (Chua et al. 1996), which is responsible for signal transduction in the hypothalamic centres influencing food intake, this may explain their hyperphagia. Obese Zucker rats are known to take much larger meals throughout the feeding period (Alingh Prins et al. 1986). In lean Wistar rats, simmondsin exerts its anorectic effect almost exclusively during the period of large meals that occurs at the start of the feeding period (Cokelaere et al. 1992; Flo et al. 1997). Thus, the greater anorectic effect of simmondsin in obese rats may also be attributed to differences in meal size. Recently, a synergistic effect between CCK and leptin on food intake reduction was described in lean mice (Barra-china et al. 1997; Matson et al. 1997). Chemical afferent vagotomy abolished this synergy, indicating a peripheral site of action of leptin through the vagus nerve (Barra-china et al. 1997). A synergistic action between CCK and leptin on food intake inhibition has not yet been described in obese Zucker rats. However, it cannot be excluded that leptin plays a role in the greater food intake reduction produced by endogenous CCK or administered simmondsin in obese rats with high leptin levels. This implies that the possible synergistic action would not depend on the long-form b-type leptin receptor that is deficient in obese Zucker rats, but on the signal transduction of short-form leptin receptors (Murakami et al. 1997) or other unknown mechanisms. Wang et al. (1997) have shown that the gastric vagus nerve contains functional leptin receptors that induce afferent signalling after leptin and/or CCK and leptin administration.

The HP pattern typically seen in free-fed lean animals shows increased HP during the dark period and a lower HP during the daytime. In free-fed obese animals, the HP pattern is flattened, so that both the nocturnal HP increase and the overall mean HP are lower than in lean animals. The data indicate that the HP pattern in rats closely parallels the activity patterns described earlier in Zucker rats by Murakami et al. (1995). Our observations are complementary to those obtained in lean and obese Zucker rats by Murakami et al. (1995) and Fukagawa et al. (1988). Murakami et al. (1995) reported that the mean daily locomotion activity and body temperature were lower in obese rats than in lean rats and that the amplitude of the nocturnal rise in body temperature and activity were significantly depressed in obese Zucker rats.

Simmondsin treatment did not disturb the typical diurnal HP pattern in lean and obese rats, but decreased the overall HP in both lean and obese rats. However, in pair-fed animals, the diurnal HP pattern was completely disturbed, as they started their food intake immediately after food presentation, during the HP measurements at 12.00 hours. Thus, it is clear that the HP pattern is correlated with the food intake pattern. The small increase in HP seen immediately after the lights were switched off indicates that HP is also linked to the locomotion activity and temperature cycle, as was to be expected, as rats normally become active immediately after the lights are switched off.

In both lean and obese rats, food intake reduction due to simmondsin treatment or pair feeding caused a significant decrease in total daily HP (Table 2). The HP:GEI value was very low in C/obese rats compared with C/lean rats. Assuming a comparable digestibility, this points to a higher energetic efficiency in C/obese rats, which will evidently cause a higher energy incorporation per unit GEI in free-feeding obese Zucker rats compared with lean Zucker rats. Our results are in line with the suggestion of Bach et al. (1981) that obese Zucker rats retain energy more efficiently than lean Zucker rats.

In S/lean and PF/lean rats, the decrease in HP, compared with C/lean rats, was proportional to the GEI decrease, suggesting that the energetic efficiency did not differ between the groups. In obese rats, however, the HP:GEI value was significantly increased in S/obese and PF/obese compared with C/obese rats. This points to a reduced energetic efficiency in obese animals on a highly restricted diet, confirmed by a greater reduction in fat deposition in restricted obese Zucker rats compared with restricted lean Zucker rats. This difference between lean and obese Zucker rats can most probably be explained by the greater simmondsin-induced food intake reduction in obese rats.

As expected due to their lower food intake, S/lean and PF/lean rats deposited less fat than lean control rats. Since leptin levels are highly correlated with body fat content (Maffei et al. 1995), it is to be expected that both S/lean and PF/lean rats will have significantly lower plasma leptin levels.

**Table 4. Plasma levels of total and HDL-cholesterol and the total:HDL cholesterol ratio in control (C), simmondsin-treated (S) and pair-fed (PF) obese and lean Zucker rats†**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total plasma cholesterol (mmol/l)</th>
<th>HDL-cholesterol (mmol/l)</th>
<th>Total:HDL-cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>C/obese</td>
<td>2.28</td>
<td>0.10</td>
<td>2.25</td>
</tr>
<tr>
<td>S/obese</td>
<td>3.96</td>
<td>0.13</td>
<td>3.36</td>
</tr>
<tr>
<td>PF/obese</td>
<td>3.28</td>
<td>0.13</td>
<td>2.92</td>
</tr>
<tr>
<td>C/lean</td>
<td>1.94</td>
<td>0.03</td>
<td>1.78</td>
</tr>
<tr>
<td>S/lean</td>
<td>2.66</td>
<td>0.08</td>
<td>2.46</td>
</tr>
<tr>
<td>PF/lean</td>
<td>2.02</td>
<td>0.05</td>
<td>1.89</td>
</tr>
</tbody>
</table>

†For details of diets and procedures, see p. 160.
levels than control lean rats. The somewhat lower (although not statistically significant so) leptin value for S/lean rats is probably related to decreased epididymal fat deposition. However, in obese rats, although simmondsin treatment resulted in the same reduction in epididymal and perirenal adipose tissue as did pair feeding, the leptin levels of S/obese rats were significantly lower than those in PF/obese rats. Further experiments are needed to determine the physiological significance of this additional decrease in leptin levels in S/obese rats.

Total cholesterol levels in both lean and obese simmondsin-treated rats were increased compared with their controls and pair-fed counterparts. The increase in total cholesterol levels in simmondsin-treated rats compared with pair-fed rats could be mostly explained by the increase in HDL-cholesterol, since the total cholesterol: HDL-cholesterol values were similar in simmondsin-treated and pair-fed rats. Total plasma cholesterol levels were similar in PF/lean and C/lean rats, but were increased in PF/obese rats compared with C/obese rats. This confirms the results of Lanza-Jacobi et al. (1986) who observed no differences in plasma levels or diurnal rhythms of cholesterol levels in meal-fed food-restricted lean Zucker rats compared with free-feeding lean Zucker rats, but did see a clear change in diurnal cholesterol rhythms in obese rats, with a peak in meal-fed food-restricted rats in the same period during which we took blood samples in our experiments. This, however, cannot explain the higher total cholesterol levels in simmondsin-treated rats compared with their pair-fed counterparts, which was mainly due to an increase in plasma HDL levels, and was a pure simmondsin-derived effect; the mechanism explaining this increase remains to be elucidated.

**Conclusions**

The food intake-reducing effect of simmondsin is more pronounced in obese Zucker rats than in their lean littermates. Except for the simmondsin-specific effects on leptin and total cholesterol values in obese Zucker rats, differences in the effects of simmondsin on growth and metabolic variables between the two types of Zucker rat can be entirely ascribed to food intake restriction.

**Acknowledgements**

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