Obesity in transgenic female mice with constitutively elevated luteinizing hormone secretion

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Kero, Jukka T., Erika Savontaus, Maarit Mikola, Ullamari Pesonen, Markku Koulu, Ruth A. Keri, John H. Nilson, Matti Poutanen, and Ilpo T. Huhtaniemi. Obesity in transgenic female mice with constitutively elevated luteinizing hormone secretion. Am J Physiol Endocrinol Metab 285: E812–E818, 2003. First published May 28, 2003; 10.1152/ajpendo.00367.2002.—Transgenic (TG) female mice, expressing a chimeric bovine luteinizing hormone (LH) β-subunit/human chorionic gonadotropin β-subunit COOH-terminal extension (bLHβ-CTP) gene, produce high levels of circulating LH and serve as a model for functional ovarian hyperandrogenism and follicular cysts. We report here that obesity is a typical feature of these female mice. The mean body weight of the bLHβ-CTP females was significantly higher than in controls at, and beyond 5 wk of age, and at 5 mo, it was 32% increased. At this age, the amount of white adipose tissue in the bLHβ-CTP females was significantly increased, as reflected by the weight difference of the retroperitoneal fat pad. In addition, the expression of leptin mRNA in white adipose tissue of the TG females was elevated about twofold. Serum leptin and insulin levels, and food intake, were also increased significantly in the TG females. Brown adipose tissue (BAT) thermogenic activity, as measured by GDP binding to BAT mitochondria, was reduced (P < 0.05). Ovariectomy at the age of 3 wk totally prevented the development of obesity. In summary, the present results show that intact female bLHβ-CTP mice are obese, have increased food consumption, and reduced BAT thermogenic activity. The weight gain can be explained partly by elevated energy expenditure as females, probably because of different regulation of the β-promoter between the two sexes (24). However, in females, expression of the transgene leads to highly elevated levels of LH. This results in increased ovarian testosterone and estradiol (E2) secretion and extensive pathological changes in the ovaries, such as enlargement, formation of multiple follicular cysts, strain-dependent granulosa cell tumors, luteomas, precocious puberty, and infertility (11, 24, 25). This mouse line thus provides a useful model of functional ovarian hyperandrogenism, associated with cystic alterations. In addition, we have recently shown that the high LH levels in the bLHβ-CTP females induce LH receptor expression in the adrenal cortex and stimulate corticosterone production, hence causing a phenotype reminiscent of Cushing’s syndrome (12). LH-stimulated ovarian estrogen production resulted in increased prolactin production, which in turn could synergize with LH in the induction of adrenal LH receptor expression and LH responsiveness. There is ample evidence in rodents about upregulation of LH receptor expression by prolactin (6, 10). In humans, chronically elevated cortisol concentration can increase body fat, as seen in Cushing’s syndrome (19, 23). The exact role of glucocorticoids in idiopathic obesity is poorly understood. Overall, the current knowledge suggests that, although circulating cortisol concentrations are normal in patients with idiopathic obesity, the secretion rates may be higher, particularly in patients with visceral adiposity (7, 18). In addition, in some, but not all cases, mutations causing dysfunctional glu-

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corticoid receptor and increased cortisol concentrations are associated with obesity (14, 26).

The objective of this study was to characterize in more detail the obesity encountered in the bLH-CTP mouse model. Herein, we describe its development and relationship with the mechanisms regulating body weight, appetite, and food intake.

MATERIALS AND METHODS

Experimental animals and treatments. The transgene expressed in the mice consists of the promoter of the bovine glycoprotein hormone α-subunit gene fused with the bovine LH β-subunit gene and followed by the coding sequence of the 24-amino acid COOH-terminal peptide of the hCG β-subunit (bLHβ-CTP; see Ref. 24). bLHβ-CTP male mice of the CD-1 genetic background were bred with C57BL/6 female mice, and the experiments were conducted using F3 and F5 generations of these crosses, unless otherwise stated. We used 4–11 mice/group in each experiment, with the nontransgenic (TG) littermates as controls. The mice were specific pathogen-free and housed 1–3 mice/cage in controlled conditions of light (12 h on and 12 h off) and temperature (22°C). They were fed with commercial mouse chow and tap water ad libitum. Food consumption was measured by weighing the food container every week before and after filling. The food intake (g/cage) was then divided by the number of mice (1–3) per cage. The mice were killed by cervical dislocation within 30 s of touching the cage, and blood samples were collected immediately by cardiac puncture. Thereafter, blood was allowed to clot overnight at 4°C and centrifuged (3000 g) at room temperature to separate serum. The sera were stored at −80°C for the experiments described previously (16). Gonadectomies of male and female mice were carried out at 0.5 kg body wt (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was injected intraperitoneally. Genotyping was accomplished using a PCR method on tail DNA as described previously (24). Gonadectomies of male and female mice were carried out at 3–4 wk of age using tribromoethanol (32) anesthesia. The hormone replacement therapies were carried out in the tenth backcross with C57BL/6 mice. These mice were ovarioectomized and implanted with E2, 5α-dihydrotestosterone (DHT), or E2 plus DHT pellets (Innovative Research of America, Sarasota, FL), using doses leading to physiological serum hormone concentrations. Magnetic resonance imaging (MRI) of the mice was performed at the Department of Radiology, Turku University Central Hospital, by a radiologist using coronal T1-weighed MR images. All procedures using mice were approved by the University of Turku Ethics Committee on Use and Care of Animals.

Measurement of serum concentrations of corticosterone, insulin, leptin, glucose, cholesterol, triglycerides, and high density lipoprotein. Serum corticosterone was measured after dichloromethane extraction by RIA, using a polyclonal rabbit antisera against corticosterone (kindly donated by Dr. R. Hampl, University of Prague, Czech Republic) and [1,2,6,7-3H]corticosterone (Amersham, Bucks, UK) as tracer (12). Serum insulin and leptin concentrations were measured by an RIA kit according to the manufacturer’s protocols (Rat Insulin and Leptin RIA kits, Linco, St. Charles, MO). Serum glucose, cholesterol, triglycerides, and high density lipoprotein (HDL) were measured in the Clinical Laboratory of Turku University Hospital using a Hitachi 917 Automatic Analyzer (Hitachi, Tokyo, Japan). Except for the case of the insulin tolerance tests, venous blood glucose was measured using a Medisense glucose meter (Medisense, Bedford, MA) with glucose electrodes.

GDP binding to the mitochondrial fraction of brown adipose tissue. Binding of [3H]GDP to the brown adipose tissue (BAT) was measured using the method by Nicholls (20) with modifications described earlier (16). Fresh BAT was minced, diluted in 250 mM ice-cold sucrose buffer, and homogenized with a Potter S homogenizer (Braun, Melsungen, Germany). The mitochondrial fraction was isolated by differential centrifugation (28). [3H]GDP binding was determined by incubating the purified mitochondria in a medium containing 100 mM sucrose, 20 mM TES, 1 mM EDTA, 10 mM choline chloride, 2 M rotenone, 0.125 mM [14C]sucrose, and 0.53 Ci/mmol of 10 μM [3H]GDP at room temperature for 10 min. Nonspecific binding was assessed in the presence of excess unlabeled GDP (1 mM). After incubation, the reaction was terminated by filtering the mixture through glass fiber filters (Thomas Scientific, Swedesboro, NJ) with a Brandel Cell Harvester (Biomedical Research Devices, Gaithersburg, MD). The GDP binding was assessed by measuring the radioactivity bound to the mitochondria by filtering the filters in a scintillation cocktail (Optic Phase “High Safe” II; FSA Laboratory Supplies, Loughborough, UK) and measuring the radioactivity in a liquid scintillation counter (LBK Wallac 1219, Turku, Finland). The amount of bound [3H]GDP, corrected by the amount of [14C]sucrose, represented the GDP binding. The protein content of the mitochondrial suspensions was assayed as described previously (22). The results are expressed as binding of GDP per milligram mitochondrial protein.

Northern hybridization. Leptin mRNA was determined in the retroperitoneal white adipose tissue (WAT) using Northern hybridization. Total RNA was isolated using the single-step acid guanidinium thiocyanate-phenol-chloroform extraction method, as previously described (4). Total RNA (20 μg) was resolved on 1% denaturing agarose gels and transferred to Hybond-N nylon membranes (RPN 303 N; Amersham, Aylesbury, UK). The membranes were prehybridized for at least 4 h at 37°C in hybridization solution containing 50% deionized formamide, 5× saline-sodium citrate, 5× Denhardt’s solution, 0.5% SDS, and 50 mg/l heat-denatured calf thymus DNA. A 25-mer oligonucleotide probe (5'-GGTCAGAGGGAGGCAGCTCTTG-3'), labeled with [γ-32P]ATP (3,000 Ci/mmol; Amersham), specific for mouse leptin mRNA, was used in the hybridization. Hybridization and washing of the membranes were performed according to the instructions of the membrane manufacturer, and the hybridization results were visualized by autoradiography using Kodak X-AR 5 film (Eastman Kodak, Rochester, NY).

Measurement of body temperature. Body temperature of the bLH-CTP mice and their non-TG-littermates was measured using a rectal probe equipped with a digital laboratory thermometer (Model BAT-12; Physitemp, Clifton, NJ). Before cold exposure, the body temperature was measured at room temperature and then at +4°C after 0.5, 2, and 22 h of exposure. The temperature was measured two to four times from each mouse at each time point.

Statistical analysis. The Statview program (version 5.01 for Windows; Abacus, Gary, NC) was used for ANOVA, followed by Fisher’s Protected LSD post hoc tests. In all statistical tests, the difference was considered significant at P < 0.05. The data are presented as means ± SE.

RESULTS

bLHβ-CTP female mice are obese and hyperphagic. The bLHβ-CTP female mice were noticeably obese (Fig. 1), and at the age of 5 mo their mean weight was 32% higher (31.0 ± 0.6 g; n = 11) than that of non-TG
The obese phenotype of the female bLHβ-CTP mice prompted us to characterize further the pattern of their weight gain. It was evident that, in bLHβ-CTP females, the amount of intra-abdominal fat was increased greatly, as noted by a 2.5-fold increase in the weight of WAT in the retroperitoneal fat pad (Table 1). This is in line with the magnetic resonance image, where increased abdominal fat was clearly seen in 8-mo-old bLHβ-CTP female mice compared with controls (Fig. 1B). The weight of the fat pad of the suprascapular BAT was also significantly increased (Table 1).

The growth curves of the females (Fig. 2) showed that the weight difference appeared at an early age. Although the birth weights of the TG and non-TG littermates showed no difference, the difference in weight gain appeared gradually during the postnatal life. A significant ($P < 0.01$) weight difference was found for the first time at 5 wk of age. No differences were found either in the nose-tail lengths at birth or at later ages between the TG and control mice (data not shown). In males, the weight curves of the TG and non-TG littermates were similar (data not shown). The weight curves shown were constructed using the F4 generation after crossing the bLHβ-CTP mice of CD-1 with the C57BL/6 strain. In the F1 generation of these crossings, both the absolute weights and weight differences between the bLHβ-CTP and control females were even higher than those found in the subsequent generations. The absolute weights were still significantly different in the F10 generation of 3-mo-old bLHβ-CTP females compared with control littermates. An increase in the weight differences was found when backcrossing the F4 generation to the CD-1 strain, suggesting that the CD-1 genetic background was responsible for the obesity (data not shown).

Obesity of bLHβ-CTP mice is associated with increased food consumption, lower thermogenesis of BAT, increased WAT leptin expression, and increased glucocorticoid levels. To evaluate the reasons for obesity of the bLHβ-CTP mice, we first measured their food consumption and BAT thermogenesis. As shown in Table 1.

### Table 1. Weights of retroperitoneal WAT and subscapular BAT pads in female and male bLHβ-CTP and non-TG mice

<table>
<thead>
<tr>
<th></th>
<th>Female bLHβ-CTP (n = 11)</th>
<th>Female non-TG (n = 4)</th>
<th>Male bLHβ-CTP (n = 13)</th>
<th>Male non-TG (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAT</td>
<td>162 ± 18*</td>
<td>64 ± 26</td>
<td>184 ± 17</td>
<td>185 ± 17</td>
</tr>
<tr>
<td>BAT</td>
<td>241 ± 15*</td>
<td>143 ± 10</td>
<td>260 ± 12</td>
<td>236 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. Units are mg. WAT, white adipose tissue; BAT, brown adipose tissue; TG, transgenic. * $P < 0.05$ compared with non-TG control.
2, the absolute food consumption of the female bLHβ-CTP mice was increased significantly, but, as expected, this was not so in the TG males. BAT thermogenic activity, as measured by a mitochondrial GDP-binding assay, was 34% reduced in the bLHβ-CTP females \((n = 11)\) compared with non-TG controls \((n = 4)\; 230 \pm 13\) vs. \(348 \pm 45\) pmol/mg mitochondrial protein, respectively, \(P < 0.05\). In line with these results, we found that the uncoupling protein-1 mRNA expression in BAT was reduced in TG females compared with non-TG controls (data not shown). Because the BAT thermogenic activity was reduced in the bLHβ-CTP female mice, we evaluated their adaptation to cold, but no difference in body temperature was found after 0.5 h, 2 h, or overnight adaptation to \(4^\circ\)C (data not shown).

We also found that the leptin mRNA expression in WAT was about twofold increased in the obese bLHβ-CTP females compared with non-TG littermates (Fig. 3). In addition, the serum corticosterone levels in female bLHβ-CTP mice were elevated significantly compared with control littermates \((492 \pm 186\) vs. \(168 \pm 110\) µg/l, respectively, \(P < 0.05\), as shown also before with this model (12). In male mice, there were no differences in corticosterone levels of the TG and control groups. As expected, the increased expression of WAT leptin mRNA resulted in significantly elevated levels of serum leptin (Fig. 4F) in transgenic females compared with controls. Serum glucose, in either randomly fed or overnight fasted 3- to 4-mo-old bLHβ-CTP females, was slightly but not significantly increased compared with non-TG controls (Fig. 4). The serum insulin levels of the bLHβ-CTP females aged 4 mo and older were increased significantly compared with controls (Fig. 4B). However, in insulin tolerance tests with 0.75 U/kg body wt insulin, no significant differences were found at either 3 or 5–6 mo of age between the bLHβ-CTP females mice and controls (Fig. 4, C and D). Serum cholesterol levels of the bLHβ-CTP mice were significantly lower than in control mice (Fig. 4E). The majority of cholesterol was of the HDL type in both groups (Fig. 4). There were no significant differences in serum triglyceride levels.

Gonadectomy of the bLHβ-CTP females reverses their LH-associated weight gain, hyperphagia, and increase in serum corticosterone. To characterize further how the abnormal ovarian function of the bLHβ-CTP mice affects their weight gain, and to analyze whether the high level of LH alone could be the causative factor, we gonadectomized groups of mice at the age of 3 wk and followed their subsequent growth rates. The data indicated that gonadectomy totally abolished the differences in body weight gain between the bLHβ-CTP females and controls. The weights at the age of 4 mo were 22.8 \(\pm 1.0\) g \((n = 4)\) and 23.2 \(\pm 0.9\) g \((n = 4)\), respectively (Fig. 5). The weights of the gonadectomized bLHβ-CTP females did not differ significantly from those of intact or gonadectomized non-TG mice. Furthermore, after gonadectomy, the differences in food consumption disappeared between the TG and non-TG female mice (data not shown).

Table 2. Average food consumption of female and male bLHβ-CTP mice and their non-TG littermates between the age of 3 to 4 mo

<table>
<thead>
<tr>
<th></th>
<th>Female bLHβ-CTP</th>
<th>Female non-TG</th>
<th>Male bLHβ-CTP</th>
<th>Male non-TG</th>
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<tbody>
<tr>
<td>((n = 11))</td>
<td>32.0 (\pm 0.6^a)</td>
<td>29.1 (\pm 0.7)</td>
<td>30.3 (\pm 0.9)</td>
<td>30.7 (\pm 1.0)</td>
</tr>
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</table>

Values are means \(\pm\) SE; \(n\), no. of mice. \(^a^P < 0.01\) compared with non-TG female.

DISCUSSION

The results of the present study indicate that chronically elevated serum LH levels can lead to obesity in...
bLHβ-CTP female mice. The obesity was associated with hyperphagia and reduced BAT thermogenesis. The cystic ovaries of these mice were shown to play a crucial role in the obesity, because gonadectomy totally reversed the excessive weight gain, hyperphagia, and increase in corticosterone secretion of the TG females. A 12 wk, replacement therapy with a physiological dose of DHT led to increased body weight and an increased amount of WAT in ovariectomized bLHβ-CTP female mice compared with ovariectomized controls, whereas E2 was anorectic and reduced both body weight and the amount of WAT.

Obesity of the LH-overexpressing females seemed to be principally the result of an increased amount of peritoneal WAT, as indicated by increased weight of the retroperitoneal fat pad. However, a part of the weight difference may have also been the result of the increased weight of the skeletal muscles caused by the anabolic effect of elevated serum androgen levels of these mice (24). As expected, also in gonadectomized control male mice, the body weights were reduced significantly compared with noncastrated controls. In addition to the anabolic effect, testosterone could be an important factor stimulating food intake of the bLHβ-CTP mice, as shown in other studies (4). In the present study, the treatment of the gonadectomized female mice with DHT alone did not significantly increase food intake, but it was able to prevent the hypophagic effect of the E2 treatment. The role of glucocorticoids in the hyperphagia of the bLHβ-CTP female mice was not specifically addressed in this study.

We have recently shown, and confirmed here, that the bLHβ-CTP female mice have high circulating corticosterone levels, express LH receptors in the adrenal cortex, and produce corticosterone in response to endogenous LH and exogenous hCG stimulation (12). Most probably, the LH-stimulated ovarian estrogen...
production caused increased prolactin production in these mice. Prolactin, in turn, has been reported to upregulate LH receptor expression in rodents (10). These data, together with the present study, show a close connection between the elevated secretion of LH and corticosterone, as well as the disturbances in energy metabolism leading to obesity. Corticosterone has also been shown to be an important regulator in other models of mouse obesity. Mice with mutations in the ob gene (ob/ob mice) encoding leptin present with severe obesity, are hyperphagic, and have decreased energy expenditure (21). These mice also have marked elevation of blood corticosterone levels, and their development of obesity has been shown to be dependent on corticosterone excess.

In addition to hormonal factors, nonshivering thermogenesis is an important regulator of energy metabolism in rodents. Thermogenesis is strongly activated when mice are exposed to cold or when they ingest an excess of calories. Several obese rodent models show impaired BAT thermogenesis, which contributes to the development of obesity (8). The present study showed that, in the BLH-CTP mice, BAT thermogenic activity was reduced despite increased food consumption. The reason for this remains unknown, but the reduced thermogenesis in the BLH-CTP females is supported by previous results showing that corticosterone decreases thermogenesis and increases lipid storage in BAT (30).

Our finding that leptin mRNA expression in WAT and serum leptin concentrations were increased in BLH-CTP females is also supported by previous studies showing a correlation between body fat and leptin concentrations (3). Furthermore, glucocorticoids have been shown to stimulate expression of the ob gene (29) and thus to have a role in elevating serum leptin levels in the BLH-CTP females. The estrogens, with approximately twofold increased levels in BLH-CTP females, might also stimulate leptin expression and release as has been suggested by other studies (2). Androgens, in contrast, are reported to inhibit leptin expression (31).

In summary, the main hormonal changes causing the obese phenotype in the BLH-CTP females is supported by the present study showing a close correlation between body fat and serum leptin levels (21). Elevated serum leptin levels have been reported in women with PCOS compared with women without this syndrome (1), but, compared with controls matched for body mass index, leptin levels did not differ significantly (17, 27).

Despite significantly increased serum insulin in the BLH-CTP female mice, compared with control mice, the insulin tolerance test used in this study did not demonstrate clear signs of insulin resistance in these transgenic mice. In human PCOS patients, however, both hyperinsulinemia and insulin resistance are common (9, 13).

In this study, the genetic background of the mice was also found to influence the extent of weight gain. Although this phenomenon was not addressed specifically, the finding is interesting, because the strain of the mice used has recently also been shown to contribute to other special phenotypic characteristics, such as ovarian tumorigenesis (11). Notwithstanding the strong element of environmental factors in obesity, genetics has also been shown to play a major role in humans (15) and mice (33). Further evaluation of the genes related to obesity in the BLHβ-CTP mice may lead to novel candidates for studying the genetic causes of weight control in humans.

In conclusion, our results demonstrate that mice having chronically elevated levels of circulating LH are obese, have increased food consumption, and have reduced thermogenic activity of BAT. The concomitant endocrine changes indicate increased ovarian estrogen and androgen production, increased pituitary prolactin secretion, and induction of LH-dependent overproduction of adrenal corticosterone. The role of ovaries in the phenotype is obvious because ovariectomy normalized the corticosterone levels and prevented the increased body weight and food consumption that occurred in intact BLHβ-CTP females. Androgen replacement therapy in the ovariectomized BLHβ-CTP females led to similar weight gain and also increased WAT as in intact TG females. However, a part of the obesity is likely the result of the LH-dependent increase in adrenal glucocorticoid secretion, which in turn is dependent on stimulation of the effect of the ovarian estrogen-prolactin link. The latter hormone apparently stimulates LH receptor expression in adrenal glands of the BLHβ-CTP females. It remains to be studied to what extent this intriguing TG mouse model will provide insight into such human diseases as, e.g., PCOS, a syndrome with similar hormonal and metabolic alterations.

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DISCLOSURES

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