Obesity in transgenic female mice with constitutively elevated luteinizing hormone secretion

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Submitted 19 August 2002; accepted in final form 21 May 2003

Kero, Jukka T., Erinika 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 androgens but is probably also contributed to the increased adrenal steroidogenesis. Hence, the bLHβ-CTP mice provide a useful model for studying obesity related to elevated LH secretion, with consequent alterations in ovarian and adrenal function.

Obesity is common in women with polycystic ovarian syndrome (PCOS; see Ref. 5). Other typical features of PCOS are elevated luteinizing hormone (LH) levels, hypersecretion of androgens, and insulin resistance (5). Causes of obesity in PCOS patients are not known, but the hormonal aberrations seen in those patients have been suggested to play an important role in the regulation of appetite and energy metabolism.

In the present study, we have used transgenic (TG) mice (bLHβ-CTP) with overproduction of LH (24). The transgene consists of the bovine α-subunit promoter fused with bovine LHβ gene, which is extended with the coding sequence for the 24-amino acid COOH-terminal peptide of the human chorionic gonadotropin (hCG) β-subunit. Adult male mice harboring the same transgene do not have constitutively elevated LH secretion as females, probably because of different regulation of the α-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-
The objective of this study was to characterize in more detail the obesity encountered in the bLHβ-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/6J 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 (300 g) at room temperature to separate serum. The sera were stored at −20°C until analyzed. In the insulin tolerance test, a dose of 0.75 U/kg body wt insulin (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/6J mice. These mice were ovariec-tomized 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-weighted 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 antiserum 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.

**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).

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>
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<tr>
<td>BAT</td>
<td>241 ± 15*</td>
<td>143 ± 10</td>
<td>260 ± 12</td>
<td>236 ± 13</td>
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</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 ± 13 vs. 348 ± 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°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 ± 186 vs. 168 ± 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 BAT thermogenic activity in bLHβ-CTP mice was increased significantly compared with non-TG females mice and 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).

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 ± 1.0 g (n = 4) and 23.2 ± 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). However, the gonadectomized non-TG males had at the age of 4 mo weights (25.1 ± 0.9 g, n = 5) that were significantly lower than in nongonadectomized male controls (31.7 ± 0.6 g, n = 4).

To specifically evaluate the role of DHT and/or E2 in hyperphagia and weight gain, ovariectomized bLHβ-CTP female and control mice were treated with DHT, E2, or with their combination between weeks 3–15 of life. The treatment of the ovariectomized bLHβ-CTP female mice with DHT increased their body weights to the same level as in the intact bLHβ-CTP mice. In contrast, E2 significantly decreased both food consumption and body weights of the mice. In gonadectomized bLHβ-CTP females, the treatment with DHT led to a significantly increased amount of WAT compared with the intact control, whereas E2 either alone or combined with DHT decreased it. In addition, E2 treatment also reduced the food consumption of gonadectomized bLHβ-CTP female mice compared with nontreated controls. The replacement therapy of gonadectomized bLHβ-CTP females with either DHT, E2, or both did not increase the corticosterone levels to those of the intact bLHβ-CTP females (results 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 (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>Food consumption, g·wk⁻¹·mouse⁻¹</td>
<td>32.0 ± 0.6b</td>
<td>29.1 ± 0.7</td>
<td>30.3 ± 0.9</td>
<td>30.7 ± 1.0</td>
</tr>
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Values are means ± SE; n, no. of mice. †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 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 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

**Fig. 4.** Serum insulin, glucose, triglycerides, high density lipoprotein (HDL) cholesterol, leptin, and response to insulin tolerance tests in 3- to 5-mo-old bLHβ-CTP females and control mice. A: blood glucose concentrations from randomly fed and overnight-fasted bLHβ-CTP females and nontransgenic littersmates. No significant differences were found between groups. B: plasma insulin concentrations from randomly fed (bLHβ-CTP, n = 6; control, n = 7) and overnight-fasted bLHβ-CTP females and control mice. C and D: insulin tolerance tests performed on 3-mo-old (C) and 5- to 6-mo-old (D) bLHβ-CTP and control mice. A dose of 0.75 U/kg body wt insulin was injected intraperitoneally. Blood samples were obtained by tail biopsy immediately before and 20–60 min after injection. The results are expressed as a percentage of blood glucose concentration before insulin injection (bLHβ-CTP, n = 6; control, n = 7). E: serum cholesterol (Chol), HDL, and triglyceride (Trigl) concentrations in bLHβ-CTP (n = 10) and control (n = 12) females. F: serum leptin levels in bLHβ-CTP (n = 10) and control (n = 12) females. *P < 0.05 and **P < 0.001, transgenic vs. nontransgenic controls.

**Fig. 5.** Weight gain of ovariectomized female bLHβ-CTP mice and their ovariectomized nontransgenic littersmates (control females, n = 4; female bLHβ-CTP, n = 5).
production 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 excess of calories. Several obese rodent models show 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 excess of calories. Several obese rodent models show 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 excess of calories. Several obese rodent models show impaired BAT thermogenesis, which contributes to the excess of calories.

Our finding that leptin mRNA expression in WAT and serum leptin concentrations was increased in bLHβ-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 bLHβ-CTP females. The estrogens, with approximated twofold increased levels in bLHβ-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 bLHβ-CTP females is probably the elevated levels of androgens and glucocorticoids. An increase in leptin expression and serum levels probably also stems from the hormonal changes but could be secondary to the increased amount of adipose tissue. A close correlation between the degree of adiposity and serum leptin levels has been reported in many studies (16). Elevated serum leptin levels have been reported in patients 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 bLHβ-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.

The skillful technical assistance of Nina Messner, Johanna Vesa, and Tarja Laiho is gratefully acknowledged. We thank Dr. Pekka Niemi for help with magnetic resonance imaging.

DISCLOSURES

This study was supported by grants from the Sigrid Juselius Foundation, The Finnish Cancer Foundation, and The Eemil Aaltonen Foundation. J. T. Kero received a fellowship from the Serono Foundation.

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