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Children with Classic Congenital Adrenal Hyperplasia Have Elevated Serum Leptin Concentrations and Insulin Resistance: Potential Clinical Implications

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Leptin is secreted by the white adipose tissue and modulates energy homeostasis. Nutritional, neural, neuroendocrine, paracrine, and autocrine factors, including the sympathetic nervous system and the adrenal medulla, have been implicated in the regulation of leptin secretion. Classic congenital adrenal hyperplasia (CAH) is characterized by a defect in cortisol and aldosterone secretion, impaired development and function of the adrenal medulla, and adrenal hyperandrogenism. To examine leptin secretion in patients with classic CAH in relation to their adrenomedullary function and insulin and androgen secretion, we studied 18 children with classic CAH (12 boys and 6 girls; age range 2–12 yr) and 28 normal children (16 boys and 12 girls; age range 5–12 yr) matched for body mass index (BMI). Serum leptin concentrations were significantly higher in patients with CAH than in control subjects (8.1 ± 2.0 *vs.* 2.5 ± 0.6 ng/ml, $P = 0.01$), and this difference persisted when leptin values were corrected for BMI. When compared with their normal counterparts, children with CAH had significantly lower plasma epinephrine (7.1 ± 1.3 *vs.* 50.0 ± 4.2, $P < 0.001$) and free metanephrine concentrations (18.4 ± 2.4 *vs.* 46.5 ± 4.0, $P < 0.001$) and higher fasting serum insulin (10.6 ± 1.4 *vs.* 3.2 ± 0.2 μU/ml, $P < 0.001$) and testosterone (23.7 ± 5.3 *vs.* 4.6 ± 0.5 ng/dl, $P = 0.003$) concentrations. Insulin resistance determined by the homeostasis model assessment method was significantly greater in children with classic CAH than in normal children (2.2 ± 0.3 *vs.* 0.7 ± 0.04, $P < 0.001$). Leptin concentrations were significantly and negatively correlated with epinephrine ($r = -0.50$, $P = 0.001$) and free metanephrine ($r = -0.48$, $P = 0.002$) concentrations. Stepwise multiple linear regression analysis indicated that serum leptin concentrations were best predicted by BMI in both patients and controls. Gender predicted serum leptin concentrations in controls but not in patients with classic CAH. No association was found between the dose of hydrocortisone and serum leptin ($r = -0.17$, $P = 0.5$) or insulin ($r = 0.24$, $P = 0.3$) concentrations in children with CAH. Our findings indicate that children with classic CAH have elevated fasting serum leptin and insulin concentrations, and insulin resistance. These most likely reflect differences in long-term adrenomedullary hypofunction and glucocorticoid therapy. Elevated leptin and insulin concentrations in patients with CAH may further enhance adrenal and ovarian androgen production, decrease the therapeutic efficacy of glucocorticoids, and contribute to later development of polycystic ovary syndrome and/or the metabolic syndrome and their complications. (*J Clin Endocrinol Metab* 87: 2114–2120, 2002)

CLASSIC CONGENITAL ADRENAL hyperplasia (CAH) due to 21-hydroxylase or 11-hydroxylase deficiency is an autosomal recessive condition in which deletions or mutations of the cytochrome P450 21-hydroxylase or 11-hydroxylase genes result in glucocorticoid and/or mineralocorticoid deficiency or excess, respectively. This leads to increased secretion of ACTH, adrenal hyperplasia, and increased production of androgens and steroid precursors before the enzymatic defect (1, 2). Current treatment is to provide adequate glucocorticoid and, when necessary, mineralocorticoid substitution to prevent adrenal crises and to suppress the abnormal secretion of androgens and steroid precursors from the adrenal cortex. The therapeutic spectrum of glucocorticoids, however, is narrow, and patients are often at risk for developing in tandem iatrogenic Cushing's syndrome and hyperandrogenism. In addition to impaired adrenocortical function, classic CAH is characterized by compromised adrenomedullary function (3). The latter is owing to developmental defects in the formation of adrenal medulla, leading to a reduction in epinephrine and metanephrine stores and decreased production of catecholamines and their metabolites.

Leptin, the product of the *ob* gene, is a 16-kDa protein secreted by differentiated white adipocytes that signals the size of sc and visceral white adipose tissue depots (4, 5). Leptin acts on the central nervous system to suppress food intake and stimulate energy expenditure by inhibiting appetite and stimulating sympathetic nervous system activity, respectively (5–8). Homozygous defects in the *ob* gene or leptin receptor gene result in obesity, whereas exogenous administration of leptin reduces food intake and body weight (7, 8). Neural, neuroendocrine, paracrine, and autocrine factors have been implicated in the regulation of *ob* gene expression and leptin secretion. Glucocorticoids and insulin increase leptin secretion (9–15), whereas androgens have the

Abbreviations: BMI, Body mass index; CAH, congenital adrenal hyperplasia; CV, coefficient of variation; HOMA, homeostasis model assessment; IR, insulin resistance; PCOS, polycystic ovary syndrome; SW, salt wasting.

opposite effect (16, 17). Catecholamines suppress *ob* gene expression and leptin secretion *in vitro* and *in vivo* via a β-adrenergic receptor mechanism (18–22), while the presence of leptin receptors in the adrenal medulla may suggest a direct interaction between leptin and adrenomedullary function (23). Obesity is associated with high serum concentrations of leptin and impaired β-adrenergic receptor-mediated lipid metabolism and thermogenesis (24, 25), implicating the importance of neuroendocrine modulators on leptin secretion.

The aim of the present study was to examine the effect of chronic adrenomedullary hypofunction, glucocorticoid treatment, and early life hyperandrogenism on leptin secretion. To this end, we determined circulating leptin concentrations in children with classic CAH and healthy children matched for body mass index, and investigated the association between leptin and various hormonal and metabolic parameters in both groups.

Subjects and Methods

Subjects

Eighteen children with classic CAH (12 boys and 6 girls; age range 2–12 yr) and 28 normal children (16 boys and 12 girls; age range 5–12 yr) were studied. Normal children were recruited to participate in the study if they were prepubertal by physical examination, had similar body mass index (BMI) to that of children with CAH, and had no evidence of any associated endocrine or other disorder. No normal subject was receiving medications. All 46 children were prepubertal and their clinical characteristics are summarized in Table 1.

In patients with CAH, clinical and endocrinologic evaluation at presentation revealed that 16 children had classic 21-hydroxylase deficiency [12 salt-wasting (SW), 4 simple virilizing], and two had 11-hydroxylase deficiency. Criteria for classification into the SW phenotype included history of salt-losing crisis with documented hyponatremia, hyperkalemia, and markedly elevated plasma renin activity. The number of adrenal crises requiring hospitalization and the associated biochemical abnormalities were obtained from parental reports and medical records. Patients in whom early virilization was diagnosed at an older age were classified as having the simple virilizing form of the disease. Diagnosis was confirmed by genetic analysis in 89% of patients (14 patients with 21-hydroxylase deficiency and 2 patients with 11-hydroxylase deficiency).

All patients with classic 21-hydroxylase deficiency were on standard doses of hydrocortisone (mean ± SD, 14.8 ± 4.2 mg/m^2 per day) given three times daily and 9α-fludrocortisone (114 ± 48 μg/d) given twice daily (26). The two patients with 11-hydroxylase deficiency were on hydrocortisone only (18.3 and 20.3 mg/m^2 per day). Six patients with classic 21-hydroxylase deficiency were also receiving GnRH agonists. Suppression of the hypothalamic-pituitary-gonadal axis in these patients was confirmed by a GnRH stimulation test at least 6 months before the study.

The study was approved by the Institutional Review Board at the National Institute of Child Health and Human Development, National Institutes of Health. Written informed consent was obtained in all cases by a parent, and assent was given by children older than 7 yr.

TABLE 1. Clinical characteristics of the 46 children

	CAH	Normal	*P*
No.	18	28	
% of females	33.3	42.9	NS
Age (yr)	7.2 ± 0.7	8.9 ± 0.3	0.03
Bone age (yr)	10.4 ± 1.0		
BMI (kg/m^2)	18.0 ± 0.8	16.8 ± 0.4	NS
Weight (kg)	30.8 ± 3.6	29.7 ± 1.4	NS
Height (cm)	125.7 ± 5.4	131.8 ± 2.1	NS
Height-age (yr)	8.0 ± 0.8	8.8 ± 0.4	NS

Values are mean ± SEM.

Methods

All children were seen early in the morning on the day of the study, and standard anthropometric measurements were obtained. Pubertal stage was determined by physical examination by one of two trained observers (D.P.M., M.W.) according to the criteria of Tanner for breast development in females and testicular development in males (27, 28).

An indwelling venous catheter for blood sampling was inserted and all subjects rested in a supine position for a minimum of 30 min before blood samples were collected. All subjects were fasting for at least 9 h before sampling. In patients with CAH, the blood samples were obtained before the administration of the morning medication.

Blood samples for measurement of leptin, insulin, glucose, testosterone, epinephrine, and free metanephrine concentrations were obtained at 0800 h. Samples were centrifuged and separated immediately after collection and were stored at −80 C until assayed.

Assays

Leptin. Serum leptin concentrations were measured using a double-antibody RIA (Esoterix Endocrinology, Calabasas, CA) with a sensitivity of 0.03 ng/ml. The intraassay coefficients of variation (CV) were 9.6% and 6.7% at serum concentrations of 1.5 ng/ml and 4.7 ng/ml, respectively. The interassay CV were 12% and 11% at serum concentrations of 0.77 ng/ml and 6.0 ng/ml, respectively.

Insulin. Insulin was measured using a two-site immunochemiluminometric assay (Esoterix Endocrinology). The sensitivity of the assay was 1.0 μU/ml. The intraassay CV were 6.2%, 6.6%, 5.2%, and 6.8% at serum concentrations of 3.5, 11, 16, and 35 μU/ml, respectively. The interassay CV were 9.8%, 7.9%, 9.3%, 7.1%, and 9.0% at serum concentrations of 4.6, 11, 18, 42, and 104 μU/ml, respectively.

Insulin resistance. Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) method as previously described: IR = insulin (μU/ml) × glucose (mmol/liter)/22.5 (29).

Epinephrine and free metanephrine. Plasma epinephrine concentrations were quantified by liquid chromatography (30). Plasma free metanephrine concentrations were determined using a different liquid chromatography procedure after extraction onto solid phase ion exchange columns (31). The lowest detection limit for both assays was 1.0 pg/ml. The intraassay CV were 3.0% for epinephrine and 3.3% for free metanephrine. The interassay CV were 9.9% for epinephrine and 5.1% for free metanephrine.

Other measurements. Serum T concentrations were measured by standard RIA (Esoterix Endocrinology). Plasma glucose concentrations were measured by the hexokinase/glucose-6-phosphate dehydrogenase assay (Boehringer, Petersburg, VA).

Statistical analysis

Nonnormally distributed data were logarithmically transformed before statistical analysis. Comparisons between two groups were performed using the two-tailed *t* test. The relation between leptin concentrations and other parameters was investigated by calculation of Pearson's correlation coefficient. Stepwise multiple linear regression analysis was used to investigate independent predictors of serum leptin concentrations in both groups of children. Independent variables tested included body mass index, serum insulin, T concentrations, plasma epinephrine and free metanephrine concentrations, and gender. Gender was included as a dichotomous variable (M = 0, F = 1). Values are expressed as mean ± SEM, unless otherwise specified.

Results

There were no significant differences in gender distribution, BMI, height, weight and height-age between children with classic CAH and normal subjects (Table 1). In normal children, there was no significant difference in BMI between

males and females (16.9 ± 0.5 *vs.* 16.6 ± 0.7). In children with CAH, however, males had significantly higher BMI than females (19.1 ± 1.1 *vs.* 16.0 ± 0.7, $P = 0.03$).

Patients with classic CAH, when compared with their normal counterparts, had significantly lower plasma epinephrine (7.1 ± 1.3 *vs.* 50.0 ± 4.2 pg/ml, $P < 0.001$) and free metanephrine (18.4 ± 2.4 *vs.* 46.5 ± 4.0 pg/ml, $P < 0.001$) concentrations (Fig. 1, A and B) and significantly higher serum leptin (8.1 ± 2.0 *vs.* 2.5 ± 0.6 ng/ml, $P = 0.01$) and insulin (10.6 ± 1.4 *vs.* 3.2 ± 0.2 μU/ml, $P < 0.001$) concentrations (Fig. 1, C and D). The differences observed in serum leptin and insulin concentrations between the two groups persisted after correction for BMI (Fig. 1, E and F). In addition, children with classic CAH had a significantly higher HOMA index than their normal counterparts (2.2 ± 0.3 *vs.* 0.7 ± 0.04, $P < 0.001$) (Fig. 1G). No significant difference in fasting plasma glucose concentrations was observed between groups (81.9 ± 2.5 *vs.* 85.4 ± 1.3 mg/dl). Leptin concentrations were significantly and negatively correlated with epinephrine and free metanephrine concentrations, both before (epinephrine: $r = -0.50$, $P = 0.001$; free metanephrine: $r = -0.48$, $P = 0.002$) and after (epinephrine: $r = -0.50$, $P = 0.001$; free metanephrine: $r = -0.47$, $P = 0.002$) correction for BMI (Fig. 2).

T concentrations were higher in children with CAH than in normal children (23.7 ± 5.3 *vs.* 4.6 ± 0.5 ng/dl, $P = 0.003$), regardless of gender. Although prepubertal by physical examination and with T concentrations in the prepubertal range, normal males had significantly lower leptin concentrations than normal females (1.5 ± 0.5 *vs.* 3.8 ± 1.1 ng/ml, $P = 0.02$). This gender difference in leptin persisted after correction for BMI ($P = 0.008$). In patients with CAH, however, gender differences in leptin concentrations were not observed.

There was no significant correlation between the dose of concurrent hydrocortisone treatment and serum leptin ($r = -0.17$, $P = 0.5$) or insulin ($r = 0.24$, $P = 0.3$) concentrations in patients with classic CAH.

Stepwise multiple linear regression analysis indicated that serum leptin concentrations were best predicted by serum insulin concentrations, BMI, and gender when all patients were considered ($r^2 = 0.73$, $P < 0.001$) (Fig. 3). In normal children, leptin concentrations were independently related to BMI and gender ($r^2 = 0.41$, $P = 0.001$), whereas in patients with CAH, the only independent predictor of leptin concentrations was BMI ($r^2 = 0.64$, $P = 0.001$). In children with CAH, insulin resistance was best predicted by BMI ($r^2 = 0.58$, $P = 0.002$).

Similar results were produced when the patients with 11-hydroxylase deficiency or those receiving GnRH agonist therapy were excluded.

Discussion

Our findings demonstrate clear differences in adrenomedullary function, and leptin and insulin secretion between children with classic CAH and normal subjects matched for BMI. When measured in the morning in a fasting and rested state, children with CAH had significantly higher serum leptin and insulin concentrations, and lower plasma epinephrine and free metanephrine concentrations than their healthy counterparts. These differences are likely to arise as a result of chronic adrenomedullary hypofunction in the former group, which may explain the elevation in both leptin and insulin concentrations, given that the secretion of both hormones is inhibited by catecholamines through β-adrenergic receptors. It is important to consider, however, that although there was no difference in BMI between the two groups, BMI may not be an accurate measure of body fat composition, and differences in body fat distribution might have contributed to the higher leptin and insulin concentrations in patients with CAH (32). It is also unknown whether diurnal variation of leptin occurs in patients with CAH, but a nocturnal rise in leptin is maintained in obesity, hyperinsulinemia (33), and Cushing's syndrome (34).

Impaired adrenomedullary function in patients with CAH was recently described (3). Patients with classic 21-hydroxylase deficiency demonstrate significantly lower (40–80%) plasma epinephrine and metanephrine concentrations as well as urinary epinephrine excretion than normal subjects. This compromise in adrenomedullary function is more pronounced in patients with the most severe, SW form of the disease and is attributed to a combination of decreased intraadrenal cortisol secretion and developmental defects in the formation of adrenal medulla (3).

The sympathetic nervous system and the adrenal medulla play an important role in the *ob* gene expression and leptin secretion. Both short- and long-term stimulation of adipose tissue β-adrenergic receptors inhibits leptin production (18–22, 35–37). In human adipose tissue cultures, incubation with isoproterenol decreases leptin mRNA levels and media concentrations of leptin in a time-dependent fashion, even in the presence of insulin or insulin and dexamethasone (38). Moreover, administration of epinephrine infusion in obese and lean subjects results in a significant reduction in leptin secretion (27%) and mRNA levels (47%), further supporting the concept of a potent inhibitory effect of β-adrenergic receptor stimulation on leptin synthesis and release (20).

Increased leptin secretion in association with long-standing sympathetic nervous system/adrenomedullary hypofunction in humans has been previously reported in Pima Indians, who also had increased incidence of obesity, IR, and diabetes mellitus type II (39, 40). The present study provides additional evidence for the involvement of the adrenal medulla in the regulation of leptin gene expression in humans. Given that leptin regulates negatively its own expression through sympathetic stimulation of β-adrenergic receptors (21, 41–43), patients with long-standing impaired adrenomedullary function may have even higher leptin concentrations than one would expect after an acute and reversible decrease of catecholamine secretion.

The elevated leptin concentrations in children with classic CAH may also be owing to their elevated insulin concentrations, compared with healthy subjects matched for BMI. Both *in vivo* and *in vitro* studies have demonstrated a dose- and time-dependent increase in leptin secretion in response to insulin (10, 14, 15, 44). Leptin, on the other hand, may inhibit insulin secretion via a direct effect on pancreatic β cells (44, 45), indicating that leptin and insulin may function as the afferent limb of a negative feedback loop that provides

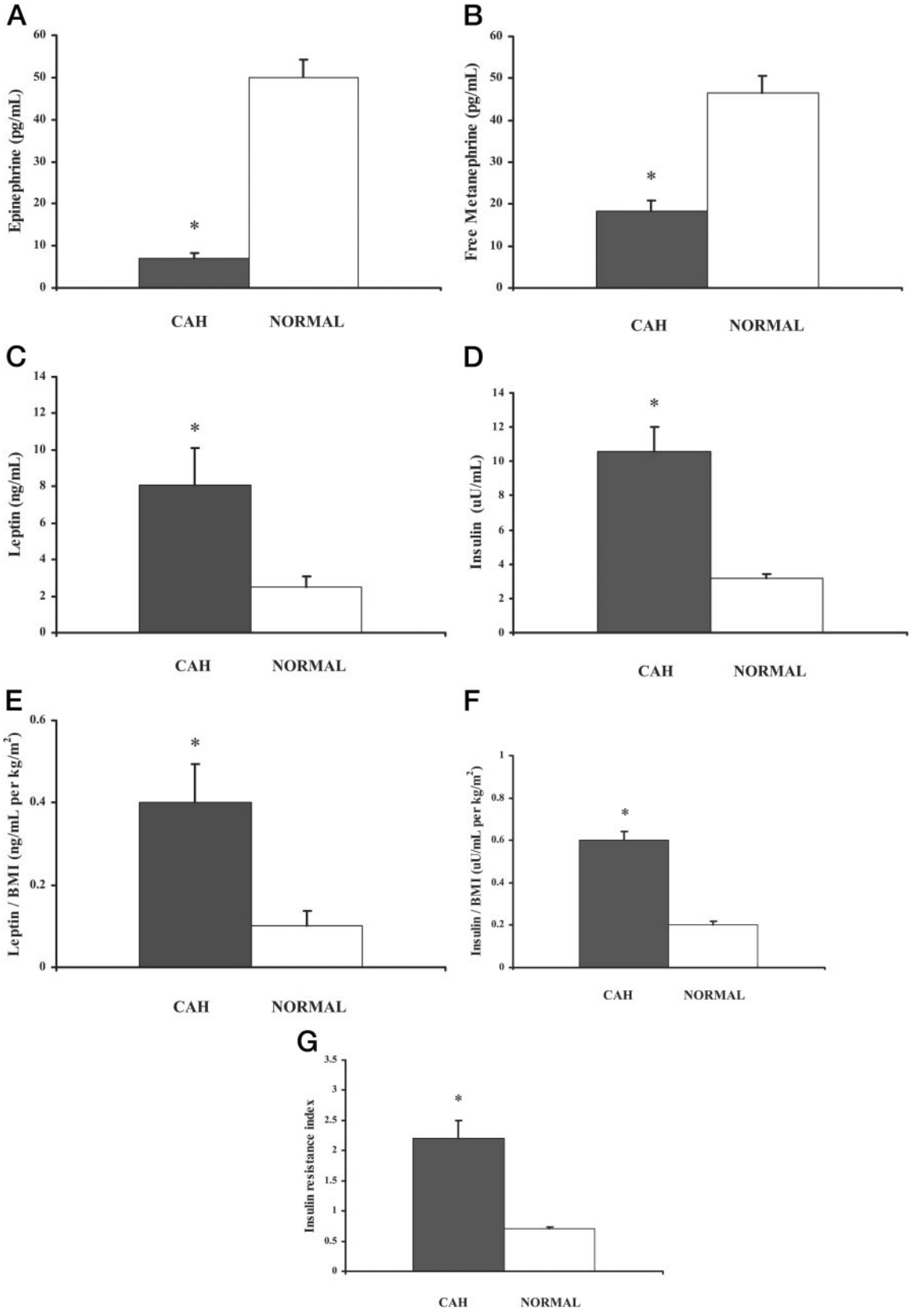

FIG. 1. Children with CAH, compared with normal children matched for BMI, had lower epinephrine (A) and free metanephrine (B) concentrations and higher leptin (C) and insulin (D) concentrations. This difference persisted when leptin and insulin values were corrected for BMI (E and F). IR, estimated using the HOMA, was significantly higher in children with CAH than normal controls (G). *Error bars* represent SEM. The *asterisks* indicate significant differences between groups.

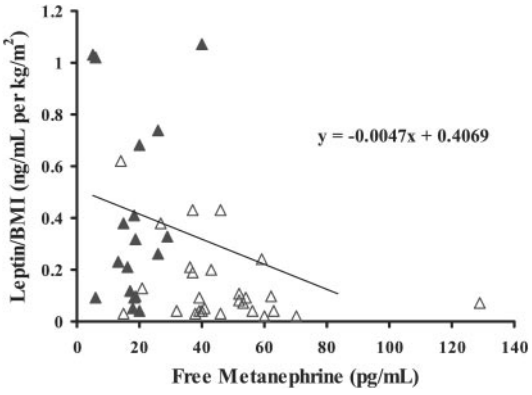

FIG. 2. Serum leptin concentrations corrected for BMI in relation to plasma free metanephrine concentrations in children with CAH (▲) and normal controls (△). There is a decline in leptin concentration with increasing catecholamine secretion.

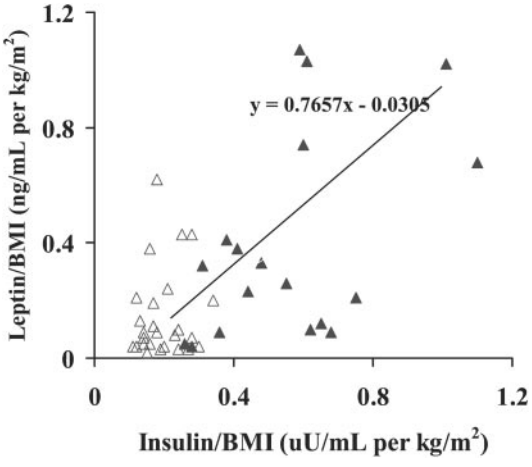

FIG. 3. Serum leptin concentrations corrected for BMI in relation to serum insulin concentrations also adjusted for BMI in children with CAH (▲) and normal controls (△). There is an increase in leptin concentration with increasing insulin concentration.

sensory input to the central nervous system about energy balance. Thus, they may interact at several levels to maintain energy homeostasis and body weight.

The significantly higher HOMA index in children with CAH, compared with normal subjects, most likely reflects the long-standing adrenomedullary hypofunction in association with intermittent hypercortisolism and/or adrenal hyperandrogenism. Although a fall in insulin sensitivity has been previously described in adults with nonclassic (late-onset) CAH (46), to our knowledge, there are no reports on alterations in insulin sensitivity/resistance in patients with the classic form of the disease.

Glucocorticoids increase *ob* gene expression and leptin secretion *in vivo*, and this effect appears to be more pronounced in obese than lean subjects (9–12). Although glucocorticoids have direct effects on the regulation of *ob* gene expression, which are independent of insulin concentrations and tissue sensitivity, they also affect leptin concentrations indirectly by inducing short- and long-term (via fat accumulation) IR and hyperinsulinism. The latter has been observed in patients with chronic hypercortisolism due to Cushing's syndrome, in which acute elevation in serum cortisol concentrations did not affect leptin secretion (47), and the observed increase in leptin concentrations was primarily associated with BMI and serum insulin concentrations (48). In our patients with CAH, the concurrent daily dose of hydrocortisone was not correlated significantly with leptin or insulin concentrations. However, this dose hardly reflects the chronic exposure of the patients to glucocorticoids, and possible interpatient differences in hydrocortisone absorption, metabolism, and/or tissue sensitivity to glucocorticoids may have obscured the effect of glucocorticoid treatment on leptin and insulin secretion in these patients.

In addition to the catecholamines and insulin, sex steroids play an important role in the regulation of leptin secretion and, along with gender differences in body composition, contribute significantly to the sexually dimorphic pattern in leptin secretion described in humans (16, 17, 49, 50). Females display a gradual rise in leptin concentrations during puberty in parallel with the rise in estrogen concentrations, whereas males show a pubertal decline in leptin concentrations (51–54). This divergent pattern of leptin secretion in males and females is primarily owing to direct effects of sex steroids on *ob* gene expression and leptin secretion (16, 17, 49) but also to an indirect effect of the BMI by shifting the relative contribution to the increase of BMI toward muscle mass in the male and fat mass in the female (52). *In vitro* studies of cultured human adipocytes revealed a suppressive effect of T and its biologically active metabolite dihydrotestosterone on leptin production, suggesting a direct effect of T at the fat cell level before its aromatization to E2 (49). In the present study, the sexual dimorphism in serum leptin concentrations was observed in normal children but not in children with classic CAH, and this may be because of the significant differences in androgen concentrations between males and females in the former but not the latter group. The fact that leptin concentrations were higher in children with CAH than in normal subjects despite their higher T concentrations indicates that insulin secretion and/or adrenomedullary function may play a more potent role than androgens in the regulation of *ob* gene expression and leptin production.

The elevated leptin and insulin concentrations in children with classic CAH may bear a number of adverse effects on the course of the disease and therefore have important implications for the management of these patients: Hyperleptinemia decreases cortisol production by decreasing the expression of steroidogenic acute regulatory protein and may predispose patients with CAH to more frequent adrenal crises (55, 56). Both hyperleptinemia and hyperinsulinism may alter the activity of enzymes participating in adrenal steroidogenesis and may result in further increases in androgen production (23, 57, 58), imposing greater difficulty on their management. Furthermore, hyperinsulinism and insulin resistance play an important role in the development of polycystic ovary syndrome (PCOS) (59), metabolic syndrome-related atherosclerotic cardiovascular disease in adult life (59, 60), and adrenal incidentalomas (61–63). Although reports on cardiovascular morbidity and mortality in patients with classic CAH are lacking, there is considerable evidence to suggest increased incidence of PCOS in females with CAH (64, 65).

We conclude that children with classic CAH have signif-

icantly higher serum leptin and insulin concentrations and increased IR index, compared with their healthy counterparts. These differences most likely reflect long-term differences in adrenomedullary function, androgen concentrations, and exposure to glucocorticoids. Both elevated leptin and insulin concentrations play a role in enhancing adrenal androgen production and may expose these patients to an increased risk for developing PCOS and the cardiovascular complications of IR. Further studies are required to determine the regulation of insulin and glucose in patients with CAH, who may benefit from prevention and treatment of their IR.

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