CLINICAL STUDY

Reduced 11 β -hydroxysteroid dehydrogenase type 1 activity in obese boys

Susanna Wiegand¹, Anna Richardt¹, Thomas Remer³, Stefan A Wudy⁴, Jeremy W Tomlinson⁵, Beverly Hughes⁵, Annette Grüters¹, Paul M Stewart⁵, Christian J Strasburger² and Marcus Quinkler²

¹Paediatric Endocrinology, Campus Virchow, Charité University Medicine Berlin, 13353 Berlin, Germany, ²Clinical Endocrinology, Charité Campus Mitte, Charité University Medicine Berlin, Charitéplatz 1, D 10117 Berlin, Germany, ³Department of Nutrition and Health, Research Institute of Child Nutrition, 44225 Dortmund, Germany, ⁴Steroid Research Unit, Center of Child and Adolescent Medicine, Justus Liebig University, 35392 Giessen, Germany and ⁵Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Queen Elizabeth Hospital, Birmingham, B15 2TH, UK

(Correspondence should be addressed to M Quinkler; Email: marcus.quinkler@charite.de)

Abstract

Objective: The incidence of childhood obesity and type 2 diabetes has reached epidemic proportions. Glucocorticoid excess causes central obesity and diabetes mellitus as seen in Cushing's syndrome. The 11 β -hydroxysteroid dehydrogenase type 1 enzyme (11 β -HSD1) regenerates active cortisol from inactive cortisone. Altered 11 β -HSD1 may cause tissue-specific Cushing's syndrome with central obesity and impaired glucose homeostasis.

Design, patients, and methods: Clinical and laboratory characteristics, and anthropometric measurements were determined in 15 male and 6 female obese pubertal children (aged 12–18 years, Tanner stages 2–5). In addition, analyses of 24-h excretion rates of glucocorticoids were also performed in 21 age-, sex-, and pubertal stage-matched non-obese children using gas chromatographic–mass spectrometric (GC–MS) analysis.

Results: 11 β -HSD1 activity (urinary tetrahydrocortisol (THF) + 5 α -THF/tetrahydrocortisone (THE) ratio) was lower in obese when compared with non-obese boys. In addition, obese children had a higher total cortisol metabolite excretion than non-obese children. 11 β -HSD1 activity was significantly related to age in lean and obese children. Standard deviation score (SDS)-body mass index did not correlate with 11 β -HSD1 activity, or with total cortisol metabolite excretion within each group. In obese children, 11 β -HSD1 activity and total cortisol metabolite excretion showed no correlation to waist-to-hip ratio, fat mass (percentage of body mass), or the homeostasis model assessment of insulin resistance index.

Conclusions: In conclusion, our findings strongly suggest that 11 β -HSD1 activity increases with age, and is reduced in obese boys. In addition, obese children have a higher total cortisol metabolites excretion suggesting a stimulated hypothalamus–pituitary–adrenal axis.

European Journal of Endocrinology 157 319–324

Introduction

Obesity is a prevalent condition and is associated with premature mortality from vascular disease. For any given body mass index (BMI), mortality is higher if fat is distributed centrally (visceral adiposity) compared with a more generalized pattern of distribution (1). This has renewed interest in factors that control adipose tissue distribution in addition to adipose tissue mass and function (2). Glucocorticoids appear to be one factor. Patients with Cushing's syndrome develop central obesity which improves with resolution of the hypercortisolism (3, 4). Although circulating cortisol (F) concentrations are normal in patients with obesity, secretion rates are higher, particularly in patients with visceral obesity (5, 6).

Recent observations indicate the importance of F metabolism in the pathogenesis of human disease processes (7, 8). 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) predominantly acts as a reductase *in vivo* by activating cortisone to cortisol regeneration and has been localized in liver and adipose tissue. 11 β -HSD1 knockout mice are unable to regenerate glucocorticoids and express a lower activation of gluconeogenesis enzymes, resulting in blunted hyperglycemia provoked by stress or obesity (9). Transgenic mice with selective overexpression of 11 β -HSD1 in white adipose tissue develop central obesity (10). Against this background, alterations in 11 β -HSD1 seem to be associated with visceral fat accumulation and obesity.

To address in detail the relationship between cortisol metabolism and body fat distribution in children, we

have undertaken a study of cortisol metabolism in a group of healthy and obese pubertal/post-pubertal children.

Subjects and methods

Between April 2003 and August 2005, we examined a cohort of 424 obese children and adolescents (aged 7–18 years) of multiethnic European origin for endocrine and metabolic abnormalities. All patients underwent a standard baseline diagnostic procedure including measuring high-density lipoprotein and low-density lipoprotein cholesterol, total cholesterol and triglyceride levels. Patients with obesity syndromes (e.g. mutations in pro-opiomelanocortin, proconvertase 1, melanocortin receptor 4, leptin) and other illnesses were excluded from this study.

From this obese cohort of 424 children, we selected a high-risk group depending on elevated liver enzymes. These were 15 boys (Tanner stages 2–5) and 6 girls (Tanner stages 3–5) aged 12–18 years. The stages of pubertal development were determined by a physician with the use of the grading system defined by Tanner for pubic hair. They were not taking any medication, did not inhale glucocorticoids, and were under a normocaloric diet before and during the study. Causes other than obesity for elevated liver enzymes were excluded.

Reference values from healthy controls were chosen from 400 healthy children and adolescents of the DONALD study (11), specifically calculated for mean ages of 15 years for boys and 15.5 years for girls. Selection criteria for matching the healthy controls (15 boys and 6 girls) to the obese cohort were: 1) pubertal Tanner stages (boys, 2–5; girls, 3–5), 2) minimum and maximum ages of 12 and 18 years respectively, and 3) a BMI-SDS of 0.0 ± 0.3 . Girls with a menstrual cycle were investigated during follicular phase of their menstrual cycle.

Overnight fasting venous blood samples were collected. Glucose was measured by the glucose oxidase method on venous whole blood immediately deproteinized with perchloric acid. The values obtained were transformed into plasma glucose values in order to calculate the insulin resistance index (homeostasis model assessment (HOMA-IR)). The HOMA-IR for insulin resistance was estimated using the formula: $\text{HOMA-IR} = (\text{fasting insulin (mIU/l)} \times \text{fasting glucose (mmol/l)}) / 22.5$. Lower HOMA-IR values indicate higher insulin sensitivity, whereas higher values indicate lower insulin sensitivity. The insulin resistance index correlates well with measures of insulin resistance obtained by euglycemic-hyperinsulinemic clamp technique, both from obese and non-obese children (12). Insulin was measured using RIA (Pharmacia). The cross-reactivity with C-peptide was 0.18% and with proinsulin was 41%. The intra-assay

variation was 5.8% for insulin, and the inter-assay variation was 7%. The lipid levels were determined by an enzymatic colorimetric test (analyser 704/717, Roche-Hitachi).

The body mass index (BMI) was calculated (the weight in kilograms divided by the square of the height in meters). The degree of obesity was quantified using Cole's least mean square method, which normalizes the BMI skewed distribution and expresses BMI as a standard deviation score (SDS-BMI) (13, 14). Body surface area (BSA) in square meters was calculated according to the modified DuBois formula: $\text{BSA} = ((\text{weight } 0.425) \times (\text{height } 0.725)) \times 0.007184$ (15).

The waist and hip circumferences were measured (only in obese children) by the same investigator, and the waist-to-hip ratio was calculated. Body composition was measured (only in obese children) with the bioimpedance device BIA-2000 M (Data Input, Hofheim, Germany) on the lying patient's right body side analyzing the frequencies of 1, 5, 10, and 50 kHz.

Pubertal status was assessed according to the criteria of Tanner. In this study, only pubertal children were assessed: boys with pubic hair and gonadal stage $\geq \text{II}$ and girls with pubic hair stage and breast stage $\geq \text{II}$. None of the subjects were taking any medication.

Patients completed a 24-h urine collection for urinary F metabolite determination. The urinary steroid metabolite profile was analyzed using a gas chromatography-mass spectrometry (GC/MS) as previously reported (16, 17), measuring free and conjugated F metabolites. Total cortisol metabolites (tetrahydrocortisone (THE), tetrahydrocortisol (THF), 5α -tetrahydrocortisol (5α -THF), α -cortolone, β -cortolone, α -cortol, and β -cortol) were used as an index of total cortisol excretion (18). The balance between 11β -HSD activities in all tissues was assessed as the ratio (tetrahydrocortisol + allo-tetrahydrocortisol)/tetrahydrocortisone ((THF + 5α -THF)/THE). The balance of 5α - and 5β -reductases was assessed by the ratio of THF/ 5α -THF (19). Relative 5α - and 5β -reduction of cortisol was also assessed by Ulick's A-ring reduction quotients 5α -THF/cortisol and THF/cortisol (20).

The study had the full approval of the local hospital ethics committee. Written informed consent was obtained from the parents and the subjects.

Statistical analysis

Statistical analyses were performed using SPSS version 13.0 for Windows (SPSS Inc, Chicago, IL, USA). Statistical analysis between boys and girls was undertaken using Student's unpaired *t*-test. Analysis of urinary steroid excretion, BMI and age was performed using ANOVA and multiple regression analysis. A *P* value < 0.05 was considered statistically significant.

Table 1 Clinical and laboratory characteristics according to sex group in obese children.

Characteristics	Obese boys (n=15)	Obese girls (n=6)
Age (years)	14.9 $\pm$ 1.6	15.4 $\pm$ 2.1
Range (years)	12–18	13–18
SDS-BMI	2.32 $\pm$ 0.65	2.73 $\pm$ 0.71
Waist-to-hip ratio	1.00 $\pm$ 0.05	0.90 $\pm$ 0.05*
Abdominal circumference (cm)	108.5 $\pm$ 14.4	108.7 $\pm$ 16.1
Fat mass (% of body mass)	30.9 $\pm$ 4.0	42.7 $\pm$ 7.9*
HOMA-IR	6.7 $\pm$ 4.2	4.8 $\pm$ 2.4
Cholesterol (mg/dl)	179 $\pm$ 26	174 $\pm$ 42
LDL cholesterol (mg/dl)	101 $\pm$ 32	103 $\pm$ 42
HDL cholesterol (mg/dl)	42 $\pm$ 6	46 $\pm$ 14
Triglycerides (mg/dl)	185 $\pm$ 116	123 $\pm$ 42
GPT/ALT (U/l)	86.8 $\pm$ 65.8	40.4 $\pm$ 14.8
GOT/AST (U/l)	51.5 $\pm$ 28.4	33.0 $\pm$ 4.6

Values are means $\pm$ s.d. BMI, body mass index. * P <0.001 compared with boys. Normal ranges: cholesterol 200 mg/dl; LDL cholesterol 160 mg/dl; HDL cholesterol 35 mg/dl; triglycerides 180 mg/dl; GPT/ALT 45 U/l, GOT/AST 50 U/l.

Results

Clinical and laboratory characteristics are presented in Table 1. There was no significant gender difference regarding the urinary (THF + 5 α -THF)/THE ratio or the urinary THF/5 α -THF ratio in obese children. In healthy non-obese children, we found a lower urinary THF/5 α -THF ratio (P <0.01) and higher 5 α -THF/F ratio (P <0.05) in boys than in girls, probably due to higher urinary 5 α -THF excretion in non-obese boys. In addition, lean boys had a higher (P <0.05) urinary cortols/cortolones ratio compared with lean girls (Table 2).

In obese boys and girls, the urinary THE excretion was significantly increased (P <0.001; P <0.05 respectively) compared with their non-obese counterparts. However, urinary (THF + 5 α -THF)/THE ratio was significantly lower (P <0.05) in obese compared with

lean boys (Table 2). The urinary cortols/cortolones ratio was significantly lower (P <0.005) in obese compared with lean boys. The sum of urinary tetrahydro metabolites (THF + 5 α -THF + THE) and the total F metabolite excretion were significantly elevated in obese compared with non-obese children independent of sex (Table 2), whereas no difference was found for urinary cortisol excretion. In addition, urinary 5 α -THF/F ratio and THF/F ratio were significantly increased in obese compared with non-obese children (Table 2).

After correction for age, there was no significant correlation between SDS-BMI and urinary (THF + 5 α -THF)/THE ratio in obese or non-obese children. However, in both groups, age was significantly correlated (P <0.001 and P <0.01) to urinary (THF + 5 α -THF)/THE ratio (Fig. 1). We did not find a significant correlation between SDS-BMI and the urinary total F metabolite excretion, the cortols/cortolones ratio, urinary THF/5 α -THF ratio, or 5 α -THF/F and THF/F ratios in obese or non-obese children.

After correction for age and SDS-BMI, urinary (THF + 5 α -THF)/THE ratio, or the urinary 5 α -THF/F and THF/F ratios showed no correlation to the waist-to-hip ratio or to the (HOMA-IR) in obese children. However, urinary (THF + 5 α -THF)/THE ratio significantly correlated positively (R^2 =0.1945; P <0.05) to the abdominal circumference in obese boys and girls (P <0.05). After correction for age and SDS-BMI, we did not find a correlation between urinary THF/5 α -THF ratio or total F metabolite excretion and metabolic parameters in obese children.

We did not find a significant difference in age, BMI, waist-to-hip ratio, abdominal circumference, fat mass (percentage of body fat), urinary (THF + 5 α -THF)/THE ratio, cortols/cortolones ratio, or total F metabolite excretion between children with a HOMA-IR >4 (n =14) compared with children with a HOMA-IR <4 (n =7).

Table 2 Clinical data and urinary steroid metabolite characteristics in obese boys and girls and in healthy lean boys and girls matched for age and pubertal Tanner stages.

Characteristics	Obese boys (n=15)	Healthy lean boys (n=15)	Obese girls (n=6)	Healthy lean girls (n=6)
BMI (kg/m ²)	31.3 $\pm$ 5.2 [‡]	19.8 $\pm$ 1.0	35.1 $\pm$ 7.5 [‡]	20.0 $\pm$ 1.0
SDS-BMI	2.32 $\pm$ 0.65 [‡]	-0.03 $\pm$ 0.12	2.73 $\pm$ 0.71 [‡]	-0.12 $\pm$ 0.16
Body surface area (m ²)	2.1 $\pm$ 0.4 [†]	1.7 $\pm$ 0.2	2.2 $\pm$ 0.3 [†]	1.7 $\pm$ 0.1
Cortisol (F; μ g/24 h)	66.2 $\pm$ 49.0	64.9 $\pm$ 21.0	48.4 $\pm$ 20.3	62.1 $\pm$ 37.3
THF (μ g/24 h)	1866 $\pm$ 1149	1255 $\pm$ 603	1316 $\pm$ 351*	923 $\pm$ 207
5 α -THF (μ g/24 h)	2346 $\pm$ 1394*	1408 $\pm$ 563 [§]	1617 $\pm$ 623*	786 $\pm$ 255
THE (μ g/24 h)	6544 $\pm$ 2986 [‡]	3379 $\pm$ 1173	4290 $\pm$ 1391*	2279 $\pm$ 1062
(THF + 5 α -THF)/THE	0.64 $\pm$ 0.18*	0.80 $\pm$ 0.20	0.73 $\pm$ 0.29	0.80 $\pm$ 0.19
Cortols/cortolones	0.28 $\pm$ 0.08 [†]	0.37 $\pm$ 0.06	0.28 $\pm$ 0.10	0.30 $\pm$ 0.04
(THF + 5 α -THF + THE)	10756 $\pm$ 5131 [†]	6042 $\pm$ 2143	7223 $\pm$ 1911*	3988 $\pm$ 1452
THF/5 α -THF	0.82 $\pm$ 0.23	0.88 $\pm$ 0.21	0.88 $\pm$ 0.27	1.24 $\pm$ 0.36
5 α -THF/F	38.4 $\pm$ 11.4 [‡]	22.3 $\pm$ 6.9 [§]	35.0 $\pm$ 11.6 [†]	14.5 $\pm$ 4.5
THF/F	30.1 $\pm$ 9.1 [†]	19.5 $\pm$ 7.0	29.9 $\pm$ 10.7*	17.4 $\pm$ 5.9
Total F metabolites (μ g/24 h)	16147 $\pm$ 7272 [†]	9027 $\pm$ 2884	11196 $\pm$ 3561*	6094 $\pm$ 1837

Values are means $\pm$ s.d. Urinary total cortisol (F) metabolites is the sum of tetrahydrocortisone (THE), tetrahydrocortisol (THF), 5 α -tetrahydrocortisol (5 α -THF), α -cortolone, β -cortolone, α -cortol, and β -cortol. * P <0.05; [†] P <0.005; [‡] P <0.001 obese compared with healthy boys or girls respectively. [§] P <0.05; ^{||} P <0.01 healthy boys compared with healthy girls.

Figure 1 Correlation between age and urinary (THF + 5 α -THF)/THE ratio. Filled squares, healthy lean children; open squares, obese children.

Discussion

Childhood obesity is rapidly increasing throughout the world (21). In addition, impaired glucose tolerance and type 2 diabetes are far more common in obese European children than previously thought (22). Endogenous cortisol may be one factor involved in fat gain during growth and in impaired glucose homeostasis. It was suggested that in obesity an increased production of adrenocorticotrophic hormone may exist and lead to an increased cortisol production (23, 24). A partial glucocorticoid resistance was suspected in obese children (25) and might be responsible for a partial and shorter suppression of the hypothalamic–pituitary axis. Only a few studies in children assessed the association between serum cortisol concentrations (26, 27) or urinary glucocorticoid excretion (28, 29) and indexes of obesity. Fasting cortisol serum concentrations have been reported to be related to insulin resistance in short-statured and growth hormone-deficient children (30), and serum cortisol concentrations were higher in obese insulin-resistant children compared with those of normal weight or obese without insulin resistance (31).

11 β -HSD1 is an important regulator of regeneration of cortisol from inactive cortisone and regulates glucocorticoid receptor activation on a pre-receptor level in liver and adipose tissue (8). 11 β -HSD activity *in vivo* is often assessed by the urinary (THF + 5 α -THF)/THE ratio. However, several studies suggested that this ratio is an inadequate indicator of 11 β -HSD1 activity (32, 33), because it is determined also by 11 β -HSD2 and A-ring reductases, and should therefore be interpreted as the balance between 11 β -HSD activities in all tissues. In adult obesity, several studies have been performed regarding 11 β -HSD activity (33, 34), but studies in obese children are still missing.

Remer *et al.* (17) described an increased urinary excretion of 6 β -hydroxycortisol and a reduced 5 α -

reductase activity in lean children with type 1 diabetes. In our study, we found a higher urinary 5 α -THF excretion and a higher relative 5 α -reduction (5 α -THF/F) in lean boys compared with lean girls. In addition, obese children of the same age showed a higher 5 α -THF excretion and a higher 5 α -THF/F ratio suggesting an increased 5 α -reductase activity. Data from adult obese patients support an increased 5 α -reductase activity (19). However, 5 α -reduction did not correlate with parameters of obesity such as fat mass, abdominal circumference, cholesterol, waist-to-hip ratio, or HOMA-IR in obese children. Obese children also showed a higher THF excretion than lean children; this was a trend in boys, but significant in girls. The relative 5 β -reduction (urinary THF/F ratio) was significantly higher in obese of both sexes than in lean children. Interestingly, healthy adult men with fatty liver present with an increased 5 β -reductase activity (33), which may alter bile acid and cholesterol metabolism. Our obese cohort consisted of children with elevated liver enzymes, most often probably due to steatohepatitis. Unfortunately, we did not measure the fat content of the liver in the obese children, which may be a confounder in cortisol metabolism. In addition, we do not have an obese control group without liver enzyme elevations.

Recently, Dimitriou *et al.* (35) reported in pre-pubertal and pubertal healthy children with normal BMI a strong correlation between age and the sum of urinary cortisol tetrahydro metabolites (THE + THF + α -THF). In their study, the (THF + 5 α -THF)/THE ratio decreased with age into puberty in healthy girls and boys with a normal BMI. In our study investigating obese and lean pubertal children, the (THF + 5 α -THF)/THE ratio increased with age. This was not due to an increased total F production, because urinary total F metabolite excretion did not correlate with age. This increased ratio probably reflects an increased endogenous production of cortisol with age in several tissues such as liver and adipose tissue. This increase is supported by the study of Reinehr & Andler (31), who showed that serum cortisol concentrations in obese children were significantly influenced by age but not by BMI. It is interesting that obese children showed an increased total cortisol metabolites excretion as well as an increased tetrahydro metabolites excretion suggesting an enhanced cortisol metabolism in obesity. This is also reflected by the increased relative 5 α - and 5 β -reduction. However, total cortisol excretion was not different in obese and lean children. A similar situation is seen in obese adults and leads to the hypothesis of a stimulated hypothalamus–pituitary–adrenal (HPA) axis in obesity due to an increased cortisol metabolism with unaltered plasma cortisol levels. However, our data were obtained from pubertal children and cannot be transferred into pre-pubertal children, due to the known effects of sex steroids on adipose tissue (36).

In our study, we were not able to show a correlation between SDS-BMI and (THF + 5 α -THF)/THE ratio or the urinary total F metabolites excretion in the obese or lean

children group. Similar results were reported by Remer *et al.* (17), who described that the (THF + 5 α -THF)/THE ratio was altered only in the subgroup of lean boys with an age 10 years, and by Dimitriou *et al.* (35), who did not find a relation between BMI and (THF + 5 α -THF)/THE ratio in healthy lean children. Nevertheless, similar to studies in adults (37), we clearly showed that obese boys, compared to lean boys, displayed a decreased urinary THF + 5 α -THF/THE ratio, indicating a shift towards E metabolites in the overall balance of 11 β -HSD activities in all tissues. This finding was supported by a decreased urinary cortols/cortolones ratio in obese compared with lean boys. In obese girls, these changes were not seen, possibly due to the small sample size of the female group. This altered (THF + 5 α -THF)/THE ratio in obese boys could explain the increase in cortisol secretion rates, through decreased cortisol half-life and subsequent activation of the HPA axis.

The observation of a decreased hepatic 11 β -HSD1 enzyme in obesity is supported by the finding that the activation of orally taken cortisone to cortisol is impaired in obese adult patients (37–39). Some studies have described decreased urinary THF + 5 α -THF/THE ratios with increasing BMI in simple obesity in adults, and this was interpreted as a decreased 11 β -HSD1 reductase activity (37, 38). However, other studies have failed to show this relationship (40–43) and, indeed, positive correlations between urinary ratio and BMI have also been described (19, 32, 39). The explanation for this discrepancy is not clear yet, but it is possible that this is due to variations in A-ring reductase activities or by tissue-specific alterations of 11 β -HSD. However, in all cases of simple obesity in adults, cortisol generation after an oral dose of cortisone is clearly reduced and suggests a reduction in hepatic 11 β -HSD1 reductase activity in obesity. However, with the known effect of glucocorticoids on adipose tissue function and distribution, it has been postulated that 11 β -HSD1 reductase activity is enhanced within omental adipose tissue and plays an important role in the pathogenesis of central obesity (8, 10).

It is worth speculating about the purpose of the hepatic downregulation of 11 β -HSD1 activity. This may be a reaction to the increased activation of cortisol in the visceral fat in obesity and, therefore, a compensatory mechanism to limit glucocorticoid exposure to key target tissues to perhaps preserve insulin sensitivity and to limit further adipogenesis. We further hypothesize that if the protective downregulation of hepatic 11 β -HSD1 is overcome or abolished, an increased activation of cortisol would have devastating effects on the metabolic situation of the individual. A higher (THF + 5 α -THF)/THE ratio in obese children was associated with a bigger abdominal circumference indicating the connection between metabolic features and cortisol metabolism. Recently, Valsamakis *et al.* (44) showed that impaired 11 β -HSD1 activity in obese adults may help preserve insulin sensitivity and prevent diabetes

mellitus. Failure to downregulate 11 β -HSD1 activity in patients with diabetes may potentate dyslipidemia, insulin resistance, and obesity. Therefore, inhibition of 11 β -HSD1 activity may represent a therapeutic strategy in patients with type 2 diabetes mellitus and obesity.

In conclusion, our study strongly suggests that (THF + 5 α -THF)/THE ratio increases with age and is reduced in obese boys. The higher total cortisol metabolite excretion probably due to increased A-ring reductase activities suggests a stimulated HPA axis in obese children similar to obese adults.

Acknowledgements

We thank all the children and their families who took part in the study.

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Received 27 March 2007

Accepted 18 June 2007