Gender Differences in Adrenal Cortex Steroid Production in SHR/N-Corpulent Rats (43813)

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Abstract. **Gender differences in adrenal steroid hormone production and serum steroid hormone levels were compared in the spontaneous hypertensive/NIH-corpulent (cp) rat, which exhibits characteristics of both obesity and non-insulin-dependent diabetes mellitus. The study demonstrated that adrenal gland size correlated with adrenal production and serum levels of steroid hormones. Obese female SHR/N-cp rats were more steroidogenic than male SHR/N-cp rats; the size of their adrenal glands was twice that of the males (70 vs 33 mg). Body weights averaged 666 g for females and 829 g for males. Obese female rats had significantly higher serum concentrations of both corticosterone (827 vs 536 ng/ml) and aldosterone (675 vs 482 pg/ml) than obese male rats. As determined by *in vitro* assay, adrenal cortex production of corticosterone (2157 vs 1435 ng/30 min) and aldosterone (13.3 vs 9.5 ng/30 min) was significantly higher in obese female than in obese male rats. Adrenal production of testosterone in the *in vitro* assay was also significantly higher for obese female than male rats; however, adrenal estrogen production in obese rats did not differ significantly. The type of carbohydrate consumed (sucrose > starch) significantly affected serum levels of corticosterone, but not aldosterone, testosterone, or estrogen. Gender differences in adrenal steroid production and serum steroid levels suggest that hyperglycemia in obese SHR/N-cp rats may be, in part, the result of excess adrenal production of steroid hormones.** [P.S.E.B.M. 1994, Vol 207]

Diabetes is associated with alterations of glucoregulatory hormones and the lack of glycemic control. Glycemic control is dependent on many factors, including pancreatic, adrenal, and liver, and insulin receptor and postreceptor functions. Evidence suggests that disruption of adrenal steroid hormones homeostasis may play a major role in the pathogenesis of both obesity and diabetes (1). Hyperglycemia is known to be associated with Cushing's syndrome (2), but the mechanism for the observed lack of glycemic control associated with Cushing's syndrome is not well understood.

Spontaneous hypertensive/NIH-corpulent (SHR/N-cp) rats exhibit both genetic obesity and non-insulin-dependent diabetes mellitus (NIDDM) (3). Obese SHR/N-cp rats are characterized by increased levels of serum insulin, triglycerides, and cholesterol and are hyperglycemic in response to oral glucose challenge. Perturbation of both carbohydrate and lipid metabolism is associated with increased levels of lipogenic and gluconeogenic enzyme activity in liver (4), increased exocrine pancreatic enzyme activity (5), and increased intestinal disaccharidase activity (6) in obese SHR/N-cp rats compared with those of lean littermates. In contrast to obese male rats, obese female rats are less hyperglycemic but exhibit markedly elevated levels of serum triglycerides (7, 8). The production of approximately twice as much corticosterone by obese female SHR/N-cp rats than by lean euglycemic

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female SHR/N-cp rats (9) is associated with hypertrophy and abnormal adrenal morphology (8).

This study investigated the effect of gender and type of carbohydrate consumed on adrenal cortex steroid hormone production and serum steroid hormone levels in both obese male and female rats. The rats were maintained for 12 weeks on diets that differed only in the type of carbohydrates (54% sucrose or cooked corn starch).

Materials and Methods

Animals. Twenty 5-week-old obese male and female SHR/N-cp rats were obtained from Dr. Carl T. Hansen, Veterinary Resources Branch, National Institutes of Health, Bethesda, MD. The animals were housed individually in stainless steel wire cages enclosed in a laminar air-flow system in a room maintained at 21°–25°C, relative humidity 40%–50%.

Diets. Rats were randomly assigned to groups that were fed two isocaloric diets containing 54% carbohydrate as either cooked corn starch or sucrose and 10% casein, 10% lactalbumin, 5.9% cellulose, 4% beef tallow, 4% lard, 4% corn oil, 4% hydrogenated coconut oil, 3.1% AIN salt mix prepared without sucrose (Teklad Test Diets, Madison, WI), and 1% vitamin fortification mix (10). Rats were given water *ad libitum* and fed the experimental diets for 12 weeks.

Physiological Measurements. Rats were maintained under quiescent conditions and were handled daily. Body weights were determined weekly. The animals were maintained on a reverse light cycle of 12-hr light (2100 to 900) and 12-hr dark (0900 to 2100). After 12 weeks on diet, between 0900 and 1100, 0.5 ml of blood was obtained from randomized fasted rats and used to determine the circulation levels of corticosterone, aldosterone, estrogen, and testosterone. This procedure was repeated at four 30-min intervals. Prior to removal of adrenal glands, rats were fasted up to 12–14 hr before and after fasting. No significant changes in body weight were observed in fasted rats. At the end of the feeding period, quiescent rats were decapitated and the adrenal glands were removed, weighed, and frozen in liquid nitrogen.

Production and Extraction of Adrenal Steroids

Media Preparation. Oxygenated (95% oxygen, 5% carbon dioxide, 30 min) Krebs Ringer bicarbonate glucose solution, containing 2.5 m*M* calcium chloride and 8.5 m*M* sodium bicarbonate buffer, was used to prepare a stock incubation media. HEPES, at a final concentration of 20 m*M*, was added to a portion of the stock incubation media and the pH was adjusted to 7.6. Just before use, the incubation medium was brought to 0.05% with bovine serum albumin (BSA), 0.14 *M* with NaCl, and Trasylol, 1000 KIU per 10 ml. The preoxygenated KREBS buffer containing 0.2% glucose, 0.06% calcium chloride, 0.05% albumin, 0.14 *M* NaCl, and Trasylol pH 7.6 (KREBGAT) constituted the incubation media (9).

Tissue Preparation. Frozen adrenal glands were trimmed of adherent fat just before the assay. Single adrenal glands were minced over a dry ice platform, until finely granular. Freshly prepared and frozen adrenal tissue can be used in this assay with comparable results.

***In Vitro* Production of Steroids.** Incubations were carried out in a hooded Dubnoff incubator at 90–100 cycles/min at 37°C. The minced adrenal tissue was placed in a 5-ml Teflon beaker containing 2 ml of preoxygenated KREBGAT incubation medium. Assay blanks were zero-time specimens (0.2 ml) removed from the nontissue supernatant, which was placed in siliconized tubes containing 15 ml methylene chloride in dry ice. Triplicate specimens from the remaining noncellular supernatant were removed at 30 and 60 min and treated in the same manner as the control to estimate steroid production. The specimens were purged with nitrogen, capped tightly, mixed, and stored at −80°C. The remaining adrenal mixture containing the cellular portion of the adrenal was then homogenized by a motor-driven tissue grinder and analyzed for protein (11).

Determination of Steroids

Adrenal Steroids. Aliquots of the methylene chloride extract were dried under N_2 and resuspended in diluent buffer from the radioimmunoassay kit. Concentrations of aldosterone, corticosterone, estrogen, and testosterone were determined by radioimmunoassay on noncellular supernatant methylene chloride extracts of adrenal-produced steroids. Data were reported as nanograms of steroid produced per 30 min.

Serum Steroids. Randomized samples (blood and adrenals) were obtained from both male and female obese rats to minimize the effects of diurnal fluctuation and estrous cycle synchronization by obese female SHR/N-cp rats. Analysis of steroids from serum followed the procedure provided in the RIA kit. The serum concentration of each steroid was determined from the standard curve and recorded as ng/ml for corticosterone and aldosterone and pg/ml for testosterone and estrogen. The cross-reactivity of corticosterone, aldosterone, estrogen, and testosterone hormones measured by the RIA kits from ICN was 0.01%.

Source of Chemicals. KREBS media, BSA (Fraction V), sodium bicarbonate, calcium chloride, methylene chloride, and HEPES disodium salt, were obtained from Sigma Chemical Co., St. Louis, MO. Steroid hormone radioimmunoassay kits were ob-

tained from Radioassay Lab Systems, ICN Biochemicals, Carson, CA. Biodegradable liquid scintillation mixture was obtained from National Diagnostics, Sommerville, NJ. Sylon-CT was obtained from Supelco, Bellefonte, PA.

Statistics. Effects were tested by two-way (gender × diet) analysis of variance (ANOVA). When necessary, ANOVA was performed by using logarithmically transformed data to satisfy the assumption of homogeneity of variance (12, 13). Differences of P values <0.05 were considered statistically significant.

Results

Adrenal Gland Wet Weight, Protein Concentration, and Glucose Tolerance. After 3 months on the diet, obese male SHR/N-cp rats weighed significantly more than obese female rats (Table I). The sucrose diet increased body weight of both male and female rats more than did the starch diet, but the difference was not significant. The adrenal wet weight of female rats was significantly greater than that of male rats (71 vs 33 mg). Part of the increase in adrenal weight was due to protein concentration of adrenals (8.2 mg for females and 5.5 mg for males). A significant diet effect (sucrose > starch) associated with the adrenal protein concentration in obese male rats was not observed in female rats.

The results from this study confirmed previous published findings (3, 6–10, 14) that showed that 1-hr glucose response values (250 mg/100 g body weight) are elevated in both obese male and female SHR/N-cp rats. Obese male SHR/N-cp rat glucose response values averaged 16.6 mmol/l compared with 14.5 mmol/l for obese female rats. There were no significant differences (10.5 mmol/l) in 1-hr glucose tolerance values of male and female lean littermates.

Steroid Concentration in Serum. The steroid hormone with the highest concentration in serum of both obese male and female SHR/N-cp rats was corticosterone (Table II). Obese female rats had a significantly higher concentration of corticosterone than did obese male rats (827 vs 536 ng/ml) and female lean littermates (753 ng/ml) (Table III). In obese female rats there was a significant diet-sex interaction with corticosterone; i.e., serum corticosterone levels were significantly higher in female rats fed sucrose than in those fed starch. The serum corticosterone level of lean female rats was significantly greater than that of lean male SHR/N-cp rats (Table III). A significant gender difference was also observed in serum concentrations of aldosterone, testosterone, and estrogen in both obese and lean rats. Obese female rats had significantly higher concentrations of aldosterone (675 pg/ml) than did obese male rats (482 pg/ml). The serum level of testosterone for obese male rats was three times higher than that of obese female rats (Table II). The serum testosterone level of lean male rats (1829 pg/ml) was significantly higher than that of male obese SHR/N-cp rats (675 pg/ml). In contrast, there was no statistical difference in serum estrogen concentration between female lean and obese SHR/N-cp rats (Table II and Table III). The serum estrogen level of obese female rats was 1.49 times that of obese male rats.

Adrenal Steroid Production. The steroid hormone produced in greatest amounts by the adrenals in obese SHR/N-cp rats was corticosterone (Table IV). Mean values for corticosterone production were 2499 ng (males) and 3905 ng (females), whereas aldosterone production was 13 ng (males) and 19 ng (females). Obese female SHR/N-cp rats synthesized and released significantly more (50% more) corticosterone and aldosterone (40% more) than did male rats. There was no significant carbohydrate effect on adrenal aldosterone or corticosterone production in obese male and female SHR/N-cp rats.

Adrenals from obese female rats synthesized significantly more testosterone than did those of male rats (Fig. 1a). However, there was no significant dietary or diet-sex interaction in adrenal testosterone production. No significant difference in adrenal pro-

Table I. Body and Adrenal Weight and Adrenal Protein of Obese SHR/N-*cp* Rats Fed Starch and Sucrose

Diet[a]	Sex	Body weight[b] (g)	Adrenal weight (mg)	Relative adrenal weight 100 g body wt	Adrenal protein (mg)
ST	M	817 ± 27	29 ± 1.4	3.5 ± 0.2	4.5 ± 0.3
SU	M	841 ± 15	37 ± 3.8	4.4 ± 0.2	6.6 ± 0.9
ST	F	660 ± 10	70 ± 7.3	10.7 ± 0.6	8.2 ± 0.5
SU	F	673 ± 33	71 ± 4.8	10.6 ± 1.0	8.2 ± 0.4
			2 × 2 ANOVA[c]		
Diet		NS	NS	NS	NS
Sex		S	S	S	S
Diet-sex		NS	NS	NS	S

[a] ST, starch; SU, sucrose; M, male; F, female.
[b] Each mean represents the average of five rats ± SEM per group.
[c] Effect significant (S) at $P < 0.05$ or nonsignificant (NS).

Table II. Serum Concentration of Steroid Hormones in Obese SHR/N-cp Rats[a]

Diet	Sex	Cort. (ng/ml)	Aldo. (pg/ml)	Test. (pg/ml)	Estr. (pg/ml)
ST	M	569 ± 20	510 ± 25	722 ± 38	273 ± 23
SU	M	504 ± 21	455 ± 38	622 ± 93	303 ± 35
ST	F	795 ± 30	652 ± 29	219 ± 6	420 ± 22
SU	F	860 ± 33	698 ± 79	217 ± 4	441 ± 12
			2 × 2 ANOVA		
Diet		NS	NS	NS	NS
Sex		S	S	S	S
Diet-Sex		S	NS	NS	NS

[a] Cort., corticosterone; Aldo., aldosterone; Test., testosterone; Estr., estrogen.

Table III. Serum Concentration of Steroid Hormones in Lean SHR/N-cp Rats[a]

Diet	Sex	Cort. (ng/ml)	Aldo. (pg/ml)	Test. (pg/ml)	Estr. (pg/ml)
ST	M	300 ± 37	500 ± 21	1770 ± 89	214 ± 12
SU	M	284 ± 31	568 ± 21	1888 ± 52	231 ± 19
ST	F	761 ± 16	629 ± 36	368 ± 25	455 ± 31
SU	F	749 ± 10	648 ± 45	396 ± 19	425 ± 46
Diet		NS	NS	NS	NS
Sex		S	S	S	S
Diet-Sex		S	NS	NS	NS

[a] See Table II for abbreviations.

duction of estrogen related to sex, diet, or diet-sex interaction was observed in obese SHR/N-cp rats (Fig. 1b).

Discussion

Insulin resistance and lack of glycemic control are associated with a number of factors, including excess adrenal steroid hormone production, especially glucocorticoids, possibly because glucocorticoids cause synergistic effects of anti-insulin activity (2, 15). Taken together these effects can result in enhanced and prolonged hyperglycemia. It is not clear what extent excess adrenal production of glucocorticoids is responsible for the above dysfunctions in obese SHR/N-cp rats and whether this lack of glycemic control is expressed in both sexes and to the same degree.

The adult female adrenal gland is larger than the adult male adrenal gland in most mammalian species. In this study, the adrenal gland of obese female rats was significantly larger than that of male obese SHR/N-cp rats. Histopathological examination of the adrenal gland of SHR/N-cp rats has shown that the adrenal glands of obese female SHR/N-cp rats are hypertrophied compared with those of male obese and lean littermates (16). Adrenal hypertrophy suggests increased corticoid production and increased serum corticosterone levels (17).

The adrenal glands of obese female SHR/N-cp rats have been shown to synthesize approximately four times more corticosterone and two times more aldosterone than those of lean female littermates (9). Furthermore, the circulation level of corticosterone of obese male and female SHR/N-cp rats was higher than that of lean littermates (9, 18).

The serum concentration and adrenal production of these steroid hormones were further magnified when sucrose rather than starch was fed as a source of dietary carbohydrate (9, 18). In the present study, obese female rats exhibited significantly higher serum levels of steroids (except testosterone) than did obese male rats. This result was reflected by a comparably higher level of adrenal steroid production of corticosterone and aldosterone. In addition, obese females, but not males, showed an enhanced response (significant only for serum corticosterone) to dietary sucrose.

Hyperinsulinemia and many other endocrine abnormalities are normalized in the obese Zucker rat by adrenalectomy (19). Previous studies have shown that the obese SHR/N-cp rats are hyperinsulinemic and hyperglycemic in response to a glucose challenge (3, 6–9, 14). In this study, obese female rats compared with obese males and lean littermates have higher circulation levels of corticosterone as well as impaired glucose response.

Gender differences in obese SHR/N-cp rats have been reported in the magnitude of hyperglycemia and hyperlipidemia (especially triglycerides) and in exocrine pancreatic activity for which the obese female rat expressed more amylase, lipase, and chymotrypsin activity than obese male rats (20, 21). These findings may suggest that the abnormal glycemic control observed in obese male and female SHR/N-cp rats are associated with different amount and form of corticosterone. Estrogens not only alter glucose and insulin response but the quantity of a carrier protein for corticosterone as well (17). Estrogen decreases glucose and insulin response (17). In addition, estrogen increases the synthesis of transcortin. Increased levels of transcortin can, in turn, increase the amount of bound corticosterone (17). Even though obese female SHR/N-cp rats produce more and have higher circulation levels of corticosterone, the amount of bioactive or unbound

Table IV. Adrenal Aldosterone and Corticosterone Production[a]

Sex	Diet	Aldosterone (ng)			Corticosterone (ng)		
		30 min (A)	60 min (B)	A + B	30 min (A)	60 min (B)	A + B
M	ST	9.4 ± 0.9	2.8 ± 0.7	12.2	1430 ± 80	1107 ± 176	2537
M	SU	9.6 ± 3.0	3.9 ± 0.7	13.5	1439 ± 78	1023 ± 215	2462
F	ST	15.1 ± 1.1	5.4 ± 1.1	20.5	2163 ± 30	1728 ± 201	3891
F	SU	12.5 ± 2.0	4.5 ± 0.6	17.0	2151 ± 36	1769 ± 128	3920
			2 × 2 ANOVA				
Diet			NS			NS	
Sex			S			S	
Diet-sex			NS			NS	

[a] Data represent total nanograms of aldosterone and corticosterone synthesized from a single adrenal gland and released into noncellular supernatant. Aliquots were removed from the supernatant at 30 min. Volume was replaced with fresh buffer and incubated for additional 60 min. Concentration of steroids was determined by RIA.

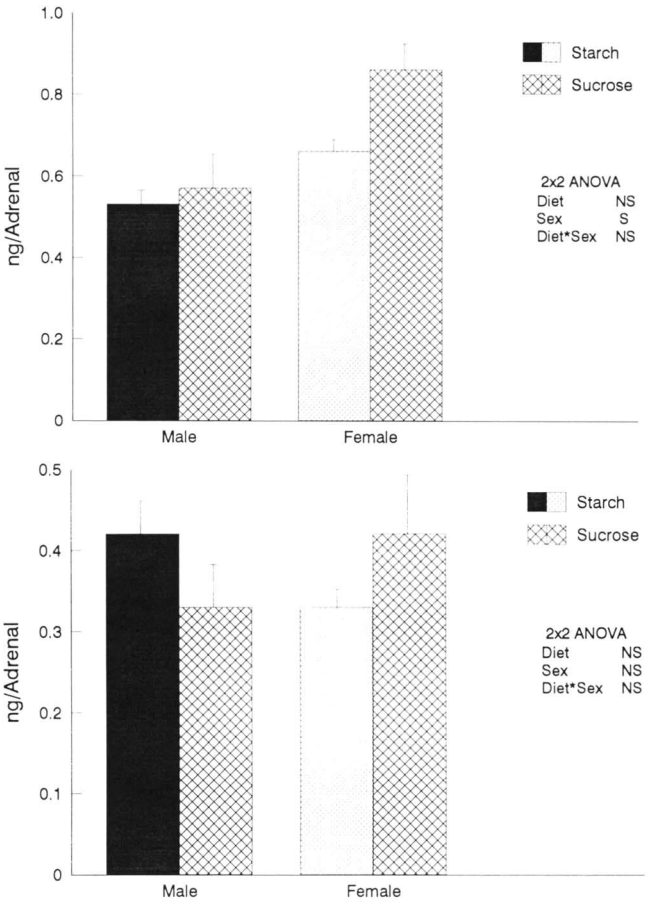

Figure 1. *In vitro* adrenal gland production of testosterone (a) and estrogen (b) in obese male and female SHR/N-cp rats fed isocaloric diets differing only in source of carbohydrate (i.e., either 54% cooked corn starch or sucrose). Minced adrenal tissue was incubated over time in KREBGAT buffer, pH 7.4, at 37°C. Aliquots of tissue-free supernatant were removed and extracted in methylene chloride. Testosterone and estrogen content of methylene chloride extracts was determined by radioimmunoassay. Data represent mean ± SEM of five rats per group. Significance, $P < 0.05$ as determined by two-way factorial ANOVA.

corticosterone is unknown. A larger proportion of the corticosterone from obese female rats may be bound to transcortin. This may explain in part why obese female rats are less hyperinsulinemic and hyperglycemic than obese male rats. The ratio of bound and free corticosterone will be the focus of future studies.

In the present study, adrenal production of testosterone was higher in obese females than in obese males. Even though adrenal production of testosterone in the obese male rat is less than that observed in obese female rats, the circulation level of testosterone is three times higher for obese males than for obese female rats. This suggests that the adrenals' role is minimal in maintaining testosterone levels. Whether or not adrenal production of testosterone by female obese rats has a role in determining fertility of the female rats is unknown. The observed lower level of circulating levels in testosterone in obese male rats compared with that of lean male rats may also contribute to the infertility observed in this strain. This has also been shown in other rodents (22). Levels of serum testosterone were lower in spontaneously diabetic BB male rats compared with control Wistar rats (23) and in chemically induced diabetic male rats (24). In addition, the testosterone levels of chemically induced diabetic female rats were higher than those of nondiabetic controls (25).

In vitro experiments suggest that free fatty acids can inhibit aldosterone secretion and that insulin reduces plasma fatty acids, which results in an increase in aldosterone production (26). Both male and female SHR/N-cp rats are hyperinsulinemic and hyperlipidemic; however, obese female rats are markedly more hypertriglyceridemic than are male obese rats (14). Although the level of plasma free fatty acids in these rats has not been reported, this mechanism may explain gender difference, observed in aldosterone production.

Although increased production of steroid hormones by the adrenal glands increases the serum levels of these hormones, elevated serum hormone levels do not rule out the possibility of a reduced rate of adrenal hormone clearance. In addition to increased steroid hormone production, obese SHR/N-cp rats may also have steroid hormone receptor or postrecep-

tor defects, which has been reported in genetically diabetic (MDB/MDB) mice (27).

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