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The Influence of Age and Fasting on Serum Hormones in the Lean and Obese Zucker Rat[1] (39729)

ROY J. MARTIN AND JOHN H. GAHAGAN

Department of Dairy and Animal Science, 301 Animal Industries Building, The Pennsylvania State University, University Park, Pennsylvania 16802

During the onset of spontaneous obesity, a series of hormonal changes occur which may or may not be essential to development of excessive adipose tissue mass. Previous workers have reported that hormone levels are altered in the obese Zucker rat when compared to the lean littermate (1–4). We have found certain metabolic changes in the obese Zucker rat which could have been induced by an endocrine imbalance (5, 6). For example, an increase in amino acid catabolism (7) and a decrease in nitrogen retention (6) may be caused by a decrease in growth hormone levels, an increase in corticosterone levels, or a combination of these two.

To initially investigate the role of the endocrine system in the development of genetically induced obesity, we wanted to characterize the changes which occur in serum levels of hormones during the dynamic phase of weight gain in the obese Zucker rat. Observations on serum levels of immunoreactive growth hormone, prolactin, thyroid-stimulating hormone, and corticosterone of lean and obese rats between 5 and 11 weeks of age are reported here.

Materials and methods. Experimental animals used in this study were from our colony of Zucker obese rats (8), which was maintained by mating heterozygotes (Fa/fa). All animals were housed in an air-conditioned room (25°) under control conditions of light (12 hr on, 12 hr off) and were fed laboratory chow (Purina) and water *ad libitum*. To study the effects of fasting on serum hormone levels, lean and obese male littermates were either fasted for 24 hr or fed. All rats were sacrificed by decapitation at the same time of day (between 9:00 and 10:00 a.m.) to minimize the effects of diurnal fluctuation of hormone levels. To study the effect of age on serum hormone changes in lean and obese rats, the rats were allowed feed and water *ad libitum* and sacrificed as described above at ages 5, 7, 9, and 11 weeks. Blood was collected from the trunk into centrifuge tubes and allowed to clot. Serum was separated by centrifugation (2000*g* for 20 min) and kept at −20° until assayed.

Insulin, TSH, prolactin, and growth hormone levels were determined using the double antibody technique of Hales and Randle (9) and modification (10) of the iodination procedure of Greenwood *et al.* (11). Materials utilized in the TSH, prolactin, and growth hormone assays were kindly supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, National Institute of Health. Standards for the TSH, prolactin, and growth hormone assays were NIAMD-Rat TSH-RP-1, NIAMD-Rat Prolactin-RP-1, and NIAMD-Rat GH-RP-1, respectively. Porcine insulin obtained from the Lilly Research Laboratories, Indianapolis, Ind. (615-D-63-10) was used as the standard for the insulin assay.

Growth hormone and prolactin used for radioiodination were dissolved in 0.01 *M* NH_4HCO_3 buffer (12) at pH 8.3. Growth hormone and prolactin standards were dissolved in 0.01 *M* NH_4HCO_3 at pH 8.3 to a dilution of 1 μg/ml and frozen. Just before use, the standards were further diluted to the desired concentration with phosphate-buffered saline containing 1% egg white (PBS-1%EW). Insulin and TSH used for radioiodination were dissolved in distilled water. Insulin and TSH standards were dissolved in PBS-1% EW.

Anti-porcine insulin (64-104-16) was ob-

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tained from Miles Laboratory, Kankakee, Ill. Anti-monkey γ-globulin used in the growth hormone assay was obtained from Antibodies Inc. Davis, Calif. Anti-guinea pig γ-globulin used in the insulin assay and anti-rabbit γ-globulin used in the TSH and prolactin assays were produced in sheep at The Pennsylvania State University.

Corticosterone was determined by competitive binding radioassay (13) using female nonpregnant rhesus monkey plasma as a source of binding globulin. Analysis of variance and unpaired t test were computed according to Steel and Torrie (14).

Results. Table I shows the effects of fasting on serum hormone levels in lean and obese rats. Insulin levels are depressed in both lean and obese rats by fasting, but still remain elevated in the obese when compared to the lean rat. Growth hormone levels were reduced by fasting in the obese rat but not in the lean rat. Prolactin and TSH were not affected by fasting in either group of animals. Corticosterone was elevated by fasting in both lean and obese rats.

Growth hormone levels increased dramatically with age in lean rats, whereas the obese rat showed only a marginal increase at 9 weeks of age (Fig. 1). Strosser and Mialhe (15) recently reported similar increases in serum GH in normal rats between 20 and 80 days of age. They concluded from their studies that metabolic clearance rate of GH was independent of serum concentration and that serum concentration depends primarily on its secretion rate from the pituitary.

Prolactin levels were higher ($P < 0.01$) in lean rats at 5 and 7 weeks of age. By 11 weeks lean and obese rats had similar levels of prolactin (Fig. 2). The prolactin levels reported here are similar to those reported in decapitated rat blood between the ages of 20 and 70 days in Holtzman male rats.

Serum levels of TSH increased with age in both lean and obese rats. At 9 weeks of age

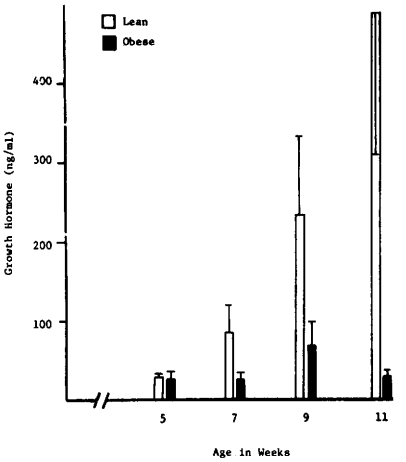

FIG. 1. Serum levels of immunoreactive growth hormone in lean and obese rats at various ages.

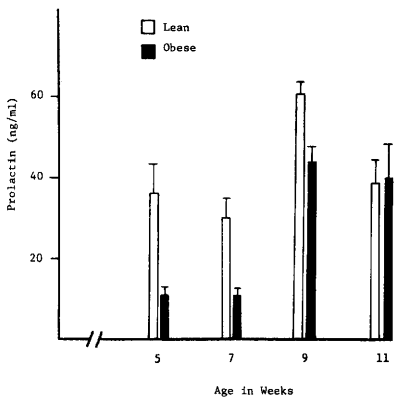

FIG. 2. Serum levels of immunoreactive prolactin in lean and obese rats at various ages.

TABLE I. THE EFFECT OF FASTING ON SERUM HORMONE LEVELS IN LEAN AND OBESE ZUCKER RATS

Hormone	Lean		Obese	
	Fed	Fasted	Fed	Fasted
Insulin (μU/ml)	120 ± 36[a]	18 ± 2*	492 ± 78	175 ± 18*
Growth hormone (ng/ml)	395 ± 149	477 ± 125	189 ± 66	30 ± 4*
Prolactin (ng/ml)	13.4 ± 2.5	15.0 ± 2.0	18.2 ± 6.4	13.2 ± 1.6
Thyroid-stimulating hormone (ng/ml)	31.87 ± 4.99	26.60 ± 1.32	28.7 ± 2.98	32.10 ± 3.49
Corticosterone (μg/100 ml)	8.80 ± 4.25	28.45 ± 10.99*	12.16 ± 4.57	38.66 ± 2.95*

[a] Mean ± SE for five animals.

* Means from fasted animal are significantly different from fed ($P < 0.05$).

serum levels of TSH were higher ($P < 0.01$) in lean rats than in obese rats (Fig. 3).

Corticosterone levels were highest at 5 weeks of age in both lean and obese rats and decreased with age (Fig. 4). Obese rats had higher levels of corticosterone at 11 weeks of age.

Discussion. Some endocrine alterations may be secondarily induced by adaptations to the obese state (17–20). This report describes hormone levels in lean and obese rats during the period of rapid weight gain and obesity onset. It is apparent that some endocrine changes observed in obese Zucker rats are caused by the obese state and are probably not directly involved in the metabolic lesions associated with this type of obesity (1, 3, 7, 21).

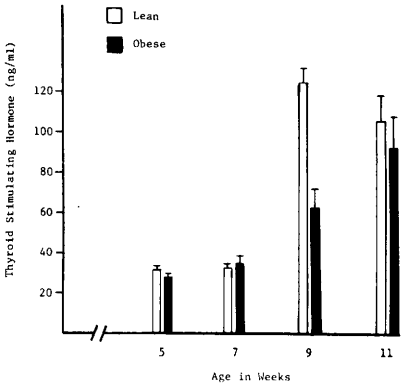

FIG. 3. Serum levels of immunoreactive thyroid-stimulating hormone in lean and obese rats at various ages.

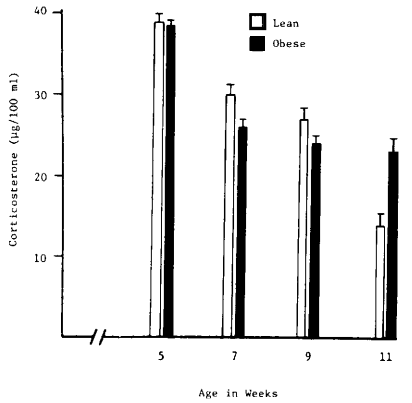

FIG. 4. Serum levels of corticosterone in lean and obese rats at various ages.

Plasma IRI levels were reduced by fasting in both lean and obese rats, and may be elevated in the genetically obese rat because of the hyperphagia associated with this syndrome. In support of this idea, Thenen and Mayer (22) showed that VMH-lesioned hyperphagic rats and the Zucker obese rats have comparable serum IRI levels. However, two types of evidence argue against this theory. We have found that pair-feeding the obese to lean rats did not eliminate hyperinsulinemia (23), and Zucker and Antoniades (1) showed that hyperinsulinemia is present after excessive accumulation of fat and that serum IRI of "fatties" fell to normal levels only when the rats have been fasted long enough to bring their body lipid down to normal.

Pituitary size is reduced in the obese rat (24) and may be the primary lesioned site causing decreased muscle growth (25), reproductive failure (26), and defective regulation of food intake in response to cold stress (27). The lower levels of growth hormone found in this study may explain the impaired muscle development (25) and decreased nitrogen retention (6) found during the dynamic phase of weight gain. Depressed serum growth hormone levels have been reported in obese hyperglycemic mice (28, 29) and hypothalamically induced obesity in rats (30) and mice (31). Elevated levels of lipids and blood glucose, often a part of the obese syndrome, may be involved in depression of serum growth hormone levels (32, 33). Growth hormone response to provocative stimulation is blunted in obese subjects but returns to normal after weight reduction (17, 18). Blackard *et al.* (32) have shown that intravenous injection of soybean oil emulsion inhibited insulin-induced plasma growth hormone elevation in monkeys. From these observations, it would appear that metabolic aberrations which result in excessive lipid synthesis and plasma lipids may produce a secondary lesion in the pituitary resulting in decreased output of growth hormone. The lower levels of growth hormone may in turn result in impaired somatomedin output (34, 35) and muscle development (25, 36) found in various types of obesities.

A role as a lipogenic factor has been im-

plicated for prolactin in the following studies: rats implanted with prolactin secretory tumors increase body weight and fat deposition (37); in the migratory white throated sparrow, prolactin was shown to act in a temporal relationship with cortisol to stimulate premigratory fattening (38); and recently we showed that, by inhibiting prolactin secretion, the lipogenic rate was decreased in the quail (39). In the present study prolactin levels were depressed during the early stages of obesity but were normal during the later stages. Similar results have been reported for the obese hyperglycemic mouse (29). While these data suggest a difference in prolactin level between lean and obese animals, more information on the diurnal pattern of prolactin and glucocorticoid levels is required for implication of these hormones as causal agents in the excessive accumulation of fats.

Hepatic amino acid catabolism and increased urinary nitrogen (6, 7) suggest that glucocorticoids may be involved in the development of obesity in the Zucker rat. Data presented in this paper suggest that an elevation in glucocorticoids occurs during the later stages of obesity and is probably a secondary adaptation to the obese condition.

York and co-workers (24) suggested from their studies that the Zucker "fatty" had irregular control of TSH secretion. In this report, plasma TSH levels were depressed in the obese at 9 weeks of age only. A depression in TSH secretion and thyroid function could lead to a decrease in basal metabolic rate and thereby increase the energy available for fatty acid synthesis. However, we observed no significant difference in energy required for maintenance of lean and obese rats (6).

This study shows changes in serum hormone levels during a period of very rapid growth. In comparison to the lean littermate, the obese rat gains weight primarily in the form of fat during this period. This genetically induced shift in dietary energy utilization may be mediated through an alteration in endocrine function. The data presented suggest a number of potential mechanisms by which muscle growth is impaired and adipose cell development is stimulated in the obese Zucker rat.

Summary. Genetically obese Zucker rats (fa/fa) and their nonobese littermates (Fa/?) were studied during the active phase of obesity onset to characterize serum levels of immunoreactive growth hormone, prolactin, thyroid-stimulating hormone, and corticosterone. The effect of fasting on serum hormone levels in lean and obese rats was also investigated. Fasting reduced insulin levels in both lean and obese rats but the fasted levels in the obese rat were still 9 to 10 times higher than the lean littermates. Growth hormone levels were lower in the obese and were reduced by fasting. In the lean rat serum growth-hormone levels increased with age (25 to 480 ng/ml); however, the obese rat showed only marginal increases (25 to 65 ng/ml) during the same period. Corticosterone levels decreased with age and were higher in the obese rat at 11 weeks of age only. Thyroid-stimulating hormone increased with age and was lower in the obese rat at 9 weeks of age. During 5 and 7 weeks of age, serum prolactin was decreased in the obese rat but was similar by 11 weeks of age. These changes are discussed in relationship to their potential role in excessive lipid deposition in genetically induced obesity.

The radioimmunoassay kits for rat growth hormone, prolactin, and TSH were supplied through the generosity of the Hormone Distribution Office, NIAMD, NIH, Bethesda, Maryland.

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