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Secretion of Calcitonin in the Genetically Obese Zucker Rat (fa/fa)[1,2] (41608)

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Abstract. Previously we found that adult Zucker fatty rats have C-cell hyperplasia and increased thyroidal calcitonin (CT) compared to lean controls. In this study we have evaluated both secretion of CT and responsiveness to CT in order to see whether they, too, were altered. Fat rats and lean littermates, 13–15 months old, were used. CT secretion was provoked by (1) feeding for 2 hr after an 18-hr fast, (2) giving pentagastrin iv, and (3) injecting $CaCl_2$ iv. CT was measured by radioimmunoassay. Responsiveness to CT was examined by giving porcine or salmon CT iv and measuring serum Ca 1–3 hr later. For CT secretion, compared to leans the fat rats showed (1) higher fasting serum Ca and CT and a greater rise in CT after feeding, (2) a similar 5- to 10-fold increase in CT after iv pentagastrin, and (3) a greater rise in both serum Ca and CT at various times between 5 min and 3 hr after iv $CaCl_2$. For CT responsiveness, fat and lean rats were equally responsive to iv CT in terms of the fall in plasma Ca 1–3 hr later. The results show that fat rats can secrete as much or more CT in response to provocative stimuli as lean rats and that they appear normally responsive to injected CT. Therefore, inability to release CT and insensitivity to CT do not underly the C-cell hyperplasia, increased thyroidal CT, and increased circulating CT in the fat rat.

The genetically obese Zucker rat (fa/fa) has been found to have a variety of endocrine and other related metabolic defects (1, 2). The development of severe obesity apparently results from the inheritance of a single autosomal recessive gene, and the rats become visibly obese by the fifth week of life (1). The obesity results, at least in part, from both hyperphagia and an increased efficiency in utilizing absorbed foodstuffs (1, 2).

Previously we found that, compared with lean littermate controls, the adult obese Zucker rate contained high levels of immunoreactive calcitonin (CT) in both the thyroid gland and the pituitary (3). Subsequent histological examination of the thyroid also revealed C-cell hyperplasia in thyroid glands taken from fat rats (4).

The experiments reported below represent an extension of our earlier studies (3, 4) and were designed to examine circulating levels of CT in lean and obese Zucker rats. Specifically, we wished to learn whether or not the C-cell hyperplasia and high thyroid content of CT might be related either to an impaired ability to release thyroidal CT or to a reduced target organ sensitivity to circulating CT. With this in mind, we measured plasma CT both basally and after stimulation of release by three experimental maneuvers reported to provoke CT secretion, namely feeding (5, 6), pentagastrin injection (7, 8), and iv administration of calcium (9, 10). Also, in order to test end-organ responsiveness to CT, we injected CT iv and compared the serum calcium-lowering (skeletal) effect of the hormone in fat rats and their lean counterparts.

Materials and Methods. *Animals.* Two shipments totaling 30 genetically obese male Zucker rats and 30 lean littermates were purchased from a colony maintained at the University of New Mexico (Alberquerque, N. Mex.) and housed individually upon receipt. Animals were 13–15 months old when they

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were used for the experiments. For results presented in Figs. 1–4, a single shipment was used. The experiments were performed in the order shown; rats were allowed to recover for 8–15 days between each experiment and were assigned randomly to treatment groups for each experiment. Except during experiments, rats were given food (Wayne lab blox) and water *ab lib.*

CT secretion. Injection and blood withdrawal were performed while animals were anesthetized deeply with ether. Blood was taken from the orbital sinus using a heparinized microhematocrit tube. Plasma was obtained by centrifugation, and samples were kept at −20°C until assayed for CT.

A. Feeding. Blood samples were obtained from rats allowed to feed *ab lib.* The rats were fasted overnight (18 hr), and a blood sample was taken. Next each rat was offered 60 g of Purina Lab Chow and allowed to eat for 2 hr at which time a second blood sample was taken and the remaining food weighed to calculate food consumption by subtraction.

B. Pentagastrin. Rats were fasted overnight, a blood sample was taken, and rats were injected iv with pentagastrin and bled again 1 min later. In one experiment all rats received a fixed dose (30 μg) of pentagastrin. In a second study rats were given 30 μg pentagastrin/100 g body weight. The synthetic peptide was kindly donated by Ayerst Laboratories (New York, N.Y.) The powder was dissolved in a small vol 0.1 *N* NH_4OH and diluted in 0.15 *M* NaCl so that each rat received a total vol of not more than 0.7 ml. Control rats received vehicle iv. All injections were delivered via a lateral tail vein.

C. $CaCl_2$. Animals were fasted overnight, a blood sample was taken, and rats were injected iv with $CaCl_2$ and bled again 5 min, 1 hr, or 3 hr later. Rats received either 0.2 *M* $CaCl_2$ (0.1 ml/100 g body weight) or an equivalent vol of 0.3 *M* NaCl as control solution. Injections were given slowly over a 1-min period via a tail vein.

CT responsiveness. Rats were fasted overnight, a blood sample was taken, and the animals were injected iv with either salmon CT or porcine CT. A second blood sample was obtained either 1 hr later (after porcine CT) or 3 hr later (after salmon CT). Both forms of CT were kindly donated by Armour Pharmaceutical Co. (Kanakee, Ill). We dissolved the powders in acidic (1 m*M* HCl) 0.15 *M* NaCl and injected 1–2 MRC mU/g body weight in a vol of 0.1 ml/100 g body weight.

Analyses. The concentration of Ca in plasma was measured by fluorometric titration using a Calcette Automatic Calcium Analyzer (Precisions Systems Inc., Sudbury, Mass.). Plasma CT was measured with a previously described homologous radioimmunoassay for rat CT (10, 11). The antiserum used was a chicken antiserum to native rat CT (final dilution 1:5000–1:10,000) and highly purified rat CT was used both for iodination with ^{125}I and as unlabeled reference standard. Bound and free hormone were separated using dextran-coated charcoal. The antiserum used recognizes largely the midregion (residues 11–23) of the CT molecule. In one experiment PTH was measured in plasma using a radioimmunoassay that measures rat PTH (12). The antiserum used was raised against bovine 1-84 PTH in a guinea pig (final dilution 1:10,000), and highly purified bovine 1-84 PTH was used for iodination and as reference standard. Purified bovine 1-84 PTH was kindly donated by Dr. Henry Keutmann, Massachusetts General Hospital, Boston, Massachusetts. The antiserum used recognizes primarily the C-terminal region (residues 65–84). Assays for both CT and PTH were conducted under so-called nonequilibrium conditions; samples and reference standards were incubated for 2–3 days, and then labeled hormone was added and the incubation allowed to continue for 2–3 days more.

Statistical analyses. Values are presented as means ± SE. Differences between mean values for fat and lean animals were assessed using an unpaired two-tailed *t* test (13). When samples were taken sequentially from each rat in a group of fat or lean rats, significance of the changes was determined using a paired two-tailed *t* test (13). In some cases where hormone levels exceeded the upper limit of detectability of the immunoassay for CT (Table III), means ± SE could not be calculated. In this case the significance of the change in plasma CT was determined using the nonparametric rank sum test of Wilcoxon (13).

Results. *CT secretion. A. Feeding.* Figure 1 shows the circulating level of CT in 19 fat rats and 20 lean controls at a time when they

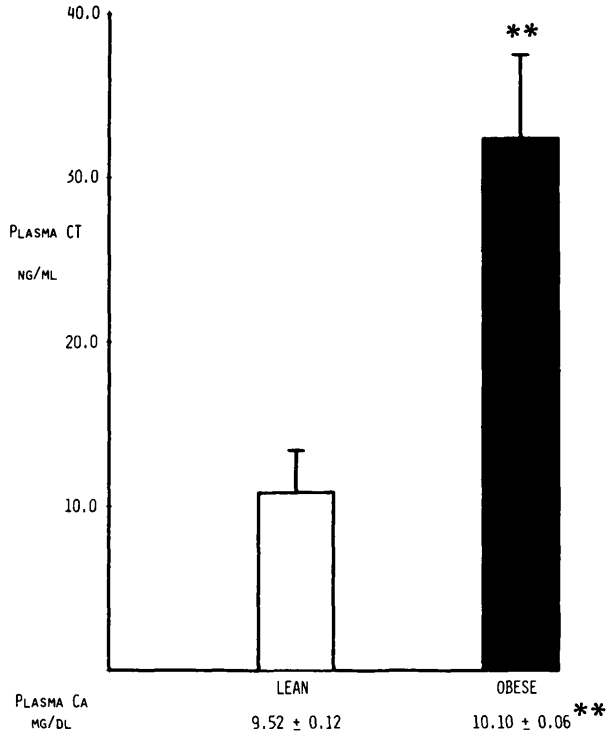

FIG. 1. Basal levels of plasma Ca and CT in lean and obese Zucker rats fed *ad lib.* In this and subsequent figures the height of each bar represents the mean, and brackets show the SE. N = 19–20 rats/group. **$P < 0.001$ vs lean.

had been fed *ad libitum* since their arrivel 8–10 months earlier. The fat rats showed both a higher resting plasma Ca concentration and a higher level of plasma CT. Subsequent experiments with these same rats are shown in Figs. 2 and 3, Table I, and Fig. 4, and the experiments were performed in the order listed. We noted that basal levels of CT declined considerably in these rats during the course of these experiments. In part this could be due to fasting [compare Fig. 1 (fed) and Fig. 2 (fasted)], but it also might be due to repeated provocation of CT release with insufficient time between experiments to allow full replenishment of thyroid CT stores by biosynthesis of new hormone.

Figure 2 shows results of an experiment where fat and lean rats were fasted overnight and then allowed to eat lab chow at will for 2 hr. Fat rats exhibited a higher fasting basal CT than the lean rats ($P < 0.005$), and they also showed a significant 2-hr elevation in CT in response to feeding. The lean rats, on the other hand, did not show a significant response at the end of the 2-hr period. Interestingly, during the 2-hr period both fat and lean rats consumed similar quantities of food (fat, 4.8 ± 0.4 g vs lean, 4.6 ± 0.4 g) despite the fact that fat rats are known to exhibit hyperphagia when fed chronically *ad lib* (1, 2).

B. Pentagastrin. Results in Fig. 3 and Table 1 illustrate that fat and lean rats both responded to pentagastrin equally well regardless of whether all rats were given a fixed dose of pentagastrin (Table I) or whether the pentagastrin was administered on a body weight basis (Fig. 3). In both cases fat and lean rats showed a three- to sixfold increase in circulating CT 1 min after pentagastrin injection. At the same time, mean values for plasma Ca actually declined slightly (Table I and Fig. 3 legends) probably at least partly because of the rapid, repetitive blood sampling.

C. $CaCl_2$. Figure 4 and Table II show results from experiments where fat and lean rats

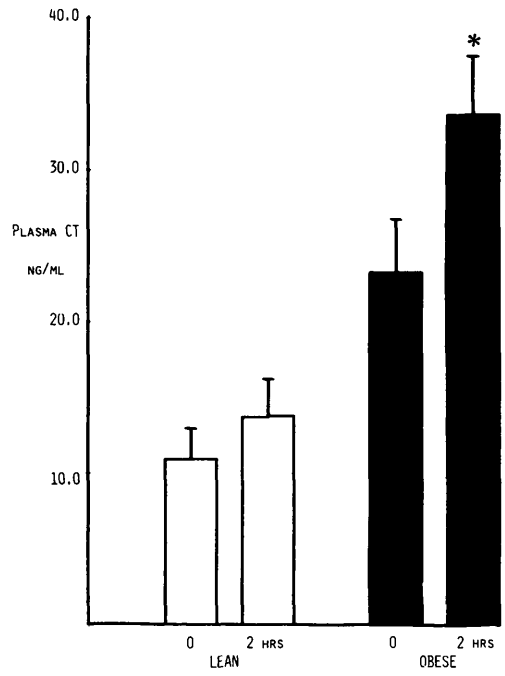

FIG. 2. Changes in plasma CT in response to feeding in lean and obese rats. Rats were fasted overnight (18 hr), bled (0 time), offered 60 g lab chow, allowed to feed *ad lib* for 2 hr, and bled again (2 hr). Lean and obese rats consumed 4.6 ± 0.4 g and 4.8 ± 0.4 g food, respectively. N = 19–20 rats/group. Plasma Ca values: 0 time = lean, 8.7 ± 0.06, fat 9.3 ± 0.08; 2 hrs = lean, 8.4 ± 0.11, fat = 8.7 ± 0.14. *$P < 0.005$ vs 0 time.

received Ca iv. Figure 4 represents animals used for experiments described above in Figs. 1–3 and Table I. Table II shows data from a second shipment of Zucker rats not previously used. Basal CT levels for lean rats were quite different for the two shipments, ~10 ng/ml (Figs. 1 and 2) vs 0.6 ng/ml (Table II). The authors have no data to explain this variability. In both experiments basal serum Ca was higher in fat rats than in lean rats and serum Ca rose higher after iv Ca in fat rats than in lean rats. Furthermore, in association with the greater rise in serum Ca after iv Ca, the fat rats showed a higher plasma CT as early as 5 min after injection of Ca (Fig. 4), and CT remained higher for up to 3 hr (Table II). Fat and lean rats did not differ in basal plasma PTH measured by our assay (Table II), and this was confirmed in six subsequent experiments (not shown). Mean values for PTH fell in both fat and lean rats reaching a nadir at 1 hr; however, statistical significance was achieved only for the fat rats at 1 hr after iv Ca (Table II).

CT responsiveness. Table III shows results of two representative experiments where lean and fat rats were injected with either porcine or salmon CT. Rats were bled either 1 hr (porcine CT) or 3 hr (salmon CT) after injection because pilot experiments showed that peak hypocalcemic responses occurred at these times. The decrease observed in plasma Ca at these times was taken as an index of the skeletal responsiveness of the rats to the hormone. As seen previously (e.g., Fig. 1 and Table II), the obese Zucker rats exhibited a higher fasting plasma Ca level than lean rats. However, at the times studied after injection of porcine or salmon CT, the plasma Ca level of fat rats had fallen to approximately the same concentration as the leans. Therefore, the actual mean percentage decrease in plasma Ca after CT was slightly greater for the fat rats than for the lean rats (porcine CT: Lean = −6.4%, Obese = −12.1%; salmon CT: Lean = −14.1%, Obese = −15.6%).

Discussion. We initiated this study to try to uncover a possible explanation for the high levels of CT we had observed earlier in the thyroid and pituitary glands of the genetically obese Zucker rat (3). We reasoned that tissue levels of CT might be high because CT release

TABLE I. EFFECT OF A FIXED DOSE OF PENTAGASTRIN (PG) IV ON PLASMA CT 1 min LATER

Group	Treatment	Plasma CT, ng/ml	
		0 time	1 min
A. Lean	Vehicle iv	1.78 ± 0.90	0.88 ± 0.27
Lean	PG iv	3.30 ± 1.38	13.06 ± 3.54*
B. Obese	Vehicle iv	3.82 ± 1.24	3.15 ± 0.96
Obese	PG iv	2.76 ± 0.76	16.16 ± 2.75**

Note. All animals were fasted 18 hr before the experiment. Rats received either pentagastrin (30 μg/0.5 ml) or 0.5 ml vehicle iv just after blood collection (0 time) and were bled again 1 min later. Plasma Ca did not differ for vehicle or PG-treated rats. Plasma Ca for all lean rats (N = 8) at 0 time was 9.4 ± 0.1 mg/dl; for fat rats Ca at 0 time was 10.0 ± 0.1 ($P < .01$ vs lean). At 1 min for leans Ca was 8.8 ± 0.2 and for fats it was 9.6 ± 0.1. Each mean value ± SE represents a group of 4 rats.

* $P < 0.05$ vs 0 time value.

** $P < 0.005$ vs 0 time value.

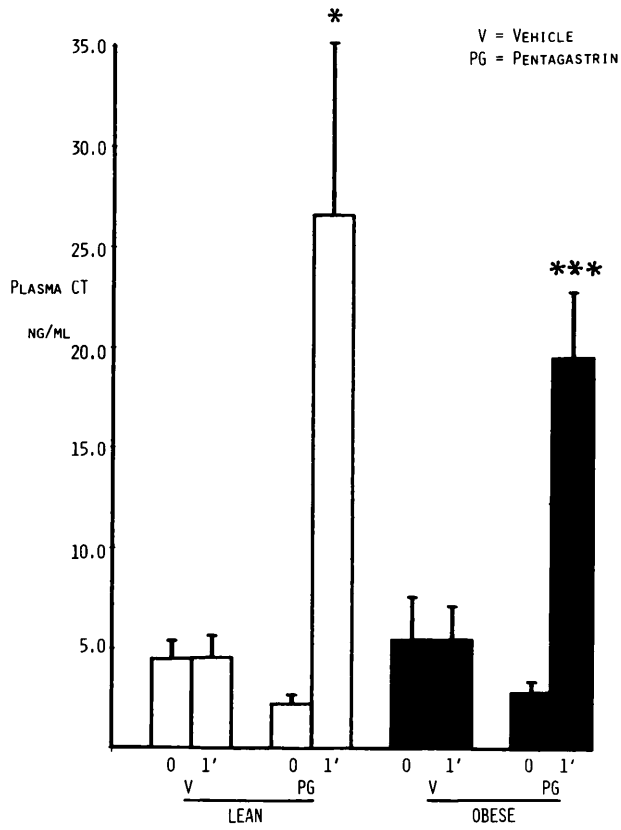

FIG. 3. Effect of pentagastrin (30 g/100 body weight, iv) on plasma CT in lean and obese rats 1 min later. Animals were fasted 18 hr before the experiment. Rats received either pentagastrin (PG) or vehicle (V) just after blood collection (0 time) and were bled again 1 min later. Plasma Ca values: lean—vehicle (N = 6)—0 time = 9.5 ± 0.3, 1 min = 9.2 ± 0.2; lean—Pg (N = 11)—0 time = 9.8 ± 0.2, 1 min = 9.6 ± 0.2; obese—vehicle (N = 6)—0 time = 10.2 ± 0.4, 1 mi = 9.9 ± 0.4; obese—Pg (N = 10)—0 time = 10.2 ± 0.2, 1 min = 9.9 ± 0.2. *P < 0.01, **P < 0.001 (vs 0 time value).

was reduced in the fat rat, allowing gland stores to increase as biosynthesis of CT outstripped secretion. A similar explanation has been invoked in the past to explain why thyroidal stores of CT increase in the chronically parathyroidectomized, hypocalcemic rat (14). However, quite the contrary, we found in this study that the fat rat has high circulating levels of CT whether fasted (Fig. 2) or fed (Fig. 1) and that fat rats appeared just as capable as lean rats in terms of being able to release CT in response to three known stimuli—feeding (Fig. 2), iv pentagastrin (Table I and Fig. 3), and iv calcium (Table II and Fig. 4). Higher basal CT levels in fat rats could be due to their tendency to have higher circulating plasma Ca levels. We were somewhat surprised to find the Zucker rat—both fat and lean—so responsive to pentagastrin (Fig. 3 and Table I). Increases in CT ranging from fourfold to as much as 100-fold (Fig. 3, Lean) were observed within 1 min after iv pentagastrin. Previously we and others have reported difficulty in provoking CT release from rat C-cells with pentagastrin (10, 11, 15). We cannot explain this discrepancy at present, but it could simply be that the Zucker strain is especially responsive.

When fat or lean rats were used repeatedly over several weeks, basal CT levels tended to fall (Figs. 1 and 2 vs Figs. 3 and 4). The reason for this fall is unknown. A remote possibility is that the 1- to 2-week interval between successive experiments may have been insuffi-

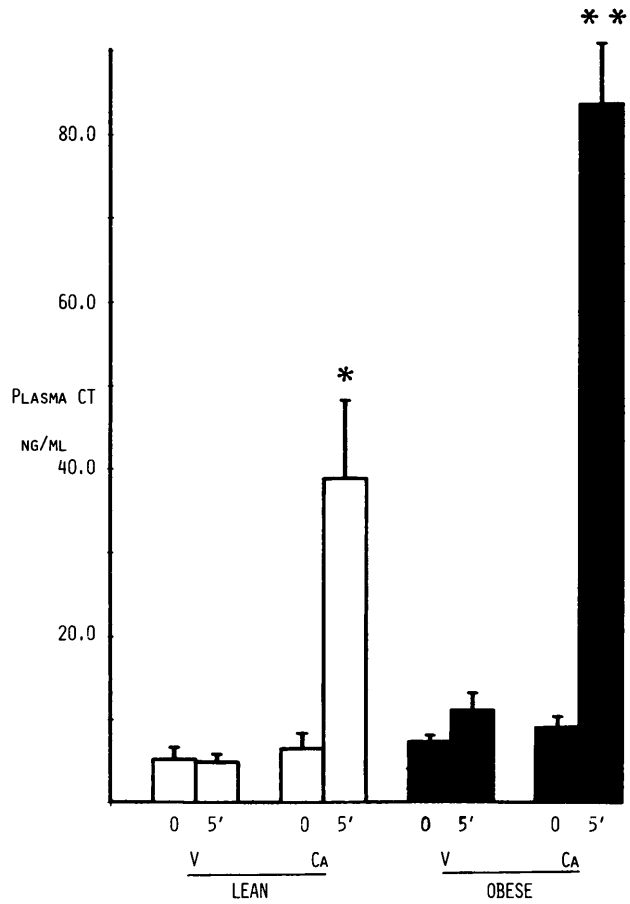

FIG. 4. Changes in plasma CT 5 min after iv Ca in lean and obese rats. Rats were fasted 18 hr before the experiment. Animals were bled at 0 time, given 0.2 *M* $CaCl_2$ (0.1 ml/100 g body weight, iv, over 1 min), and bled again 5 min later. Controls received 0.3*M* NaCl (0.1 ml/100 g) as vehicle (V). Plasma Ca values: lean—vehicle (N = 7)—0 time = 9.7 ± 0.2, 5 min = 9.2 ± 0.1; lean—Ca (N = 10)—0 time = 10.2 ± 0.1, 5 min = 13.0 ± 0.4**; obese—vehicle (N = 8)—0 time = 10.4 ± 0.2, 5 min = 9.8 ± 0.1; obese—Ca (N = 7)—0 time = 10.1 ± 0.2, 5 min = 16.7 ± 0.4**. *P < 0.005, **P < 0.001 (vs 0 time value).

cient time to allow complete replenishment of gland stores by biosynthesis. Measurement of tissue stores before and after provocation of release would be required to address this hypothesis. There is published evidence suggesting that gland stores can be depleted when rats are stimulated to release CT by making them severely hypercalcemic (14).

In the present study we also noted that fat rats exhibited a higher plasma calcium level than lean controls after receiving Ca iv (Fig. 4 and Table II). Since the fat rats were dosed with Ca on a body weight basis, they received a higher total dose of Ca than lean rats. However, fat rats differ in weight largely because of increased body fat not because of an increase in lean body mass (1, 2). Therefore, the most likely explanation for the higher plasma calcium we observed is that a higher dose of $CaCl_2$ was distributed in a similar volume of plasma and extracellular fluid. We recognize, however, that other possible explanations exist and that additional experiments will be required to address this issue.

In this study, we also sought to determine whether or not fat rats were as responsive to CT as lean controls. We did this by injecting CT and observing the hypocalcemic response

TABLE II. CHANGES IN PLASMA Ca, CT, AND PTH AT VARIOUS TIMES AFTER IV Ca

Group	Plasma conc.	0 time	5 min	1 hr	3 hr
A. Lean	Ca, mg/dl	9.3 ± 0.1	13.3 ± 0.3**	10.7 ± 0.3**	9.3 ± 0.4
	CT, ng/ml	0.6 ± 0.1	>63**	44 ± 12*	12 ± 6
	PTH, ng/ml	3.0 ± 0.6	2.7 ± 0.5	2.0 ± 0.2	2.6 ± 0.3
B. Obese	Ca, mg/dl	10.0 ± 0.1	15.8 ± 0.6**	12.2 ± 0.3**	10.0 ± 0.2
	CT, ng/ml	23 ± 6	>63**	>63**	56 ± 4*
	PTH, ng/ml	2.5 ± 0.5	2.1 ± 0.5**	1.4 ± 0.3*	2.1 ± 0.4

Note. All animals were fasted 18 hr before the experiment. Rats were bled at 0 time and then given 0.2 *M* $CaCl_2$, 0.1 ml/100 g body weight, iv over 1 min. Additional blood samples were taken at the times shown. $N = 6$ for each group of fat and lean rats.

* $P < 0.05$ vs 0 time value.

** $P < 0.01$ vs 0 time value.

1–3 hr later. It is well established that the intensity of the hypocalcemia is a reflection of the extent of the skeletal response to CT in terms of inhibition of efflux of Ca from bone to blood (16, 17). We hypothesized that fat rats might be less responsive to CT for two reasons: (i) previous work with different strains of rats had showed that those strains having the highest thyroidal stores of CT were less responsive to injected CT (18), and (ii) since fat rats have chronically high circulating levels of CT, it was reasonable to expect that skeletal receptors for CT might be down-regulated or densensitized. However, our results (Table III) gave no indication of an impairment in fat rats in terms of the extent of hypocalcemia after iv injection of CT. However, we recognize fully that our findings are limited and that more subtle changes might be revealed by more extensive dose and time studies with iv CT. Also, direct measurement of skeletal CT receptor numbers and affinity in fat and lean rats would be required before firm conclusions could be made. At present, however, we have no evidence for an impaired skeletal response to CT in the fat rat.

High levels of CT in the pituitary, thyroid, and blood of the genetically obese Zucker rat remain an enigma. Likewise, we presently do not know why these rats exhibit hypercalcemia and C-cell hyperplasia or whether other obese animal models show these changes in Ca and CT. Further studies will be required to explore mechanisms underlying these changes and to see whether other mammalian species, including man, also show changes in CT associated with obesity.

TABLE III. FALL IN PLASMA Ca AFTER IV INJECTION OF PORCINE CT OR SALMON CT

Exp	Group	Initial plasma Ca, mg/dl	Final plasma Ca, mg/dl
1. Porcine CT	Lean	9.3 ± 0.1	8.7 ± 0.1*
	Obese	9.9 ± 0.2	8.7 ± 0.1*
2. Salmon CT	Lean	9.2 ± 0.1	7.9 ± 0.2*
	Obese	9.6 ± 0.1	8.1 ± 0.1*

Note. All animals were fasted 18 hr before the experiment. Rats were bled, injected with CT, and bled again 1 hr (Expt 1) or 3 hr (Expt 2) later. Porcine CT = 1 MRC mU/g body weight; salmon CT = 2 MRC mU/g body weight. $N = 6$ for each group in Expt 1; $N = 4$ for each group in Expt 2. Endogenous (rCT) levels (ng/ml) for rats in Expt 1 were: before injection—lean = 3.39 ± 0.93, obese = 7.63 ± 1.75 ($P = 0.05$); after injection—lean = 0.44 ± 0.15, obese = 0.85 ± 0.47. Endogenous CT levels were not measured in Expt 2.

* $P < 0.01$ vs initial plasma Ca.

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