Regulation of Water Balance in Genetically Obese Rats[1] (35368)

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(Introduced by D. H. Solomon)

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During studies on the hyperphagia of genetically obese rats (unpublished data), it was noted that they produced excessive volumes of urine. This paper describes the results of experiments designed to identify the reason(s) for this diuresis.

Materials and Methods. Fatty rats and their lean siblings were purchased from Dr. L. M. Zucker.[2] Lesions were placed in the ventromedial nuclei of the hypothalamus of lean rats as previously described (1), and only those animals which displayed hyperphagia and rapid weight gain were used. Animals were approximately 3 to 4 months old at the time of these experiments and were maintained in individual stainless steel metabolism cages. Food was available *ad libitum* or was restricted to 14 g of ground chow/day. Water was freely available except where stated in the text.

Food intakes and urine volumes were measured daily between 9:00 and 10:00 a.m. Daily water intake was measured gravimetrically. Allowance was made for water spillage in transference of water bottles to the balance by utilizing two cintrol bottles.

Urine was collected under mineral oil. Aliquots were diluted four times and the osmolality was measured on an Advanced osmometer. Freshly prepared standards of fourfold dilution were used to verify the linearity and accuracy of the readings which showed a maximum variation of ±3%. Urine protein was measured by the biuret method (2). Pitressin, 0.5 U/day (pitressin tannate in arachis oil, Parke, Davis Company), was injected intramuscularly for 5 days. Control animals received injections of vehicle only.

Kidneys were dissected free, cleaned of surrounding fat, fixed in 10% formalin, and sections were stained with hematoxylin and eosin. The results were expressed as mean ± SE. Significance was calculated using the Student's *t* test.

Results. It was initially thought that the increased urine production of genetically obese rats fed *ad libitum* was the result of larger water intake in response to hyperphagia with increased solute ingestion. However, when the food intake of genetically obese rats was restricted to the same amount of chow per day as their lean littermates, urine volumes were still much greater than those of lean animals (Fig. 1). The feeding

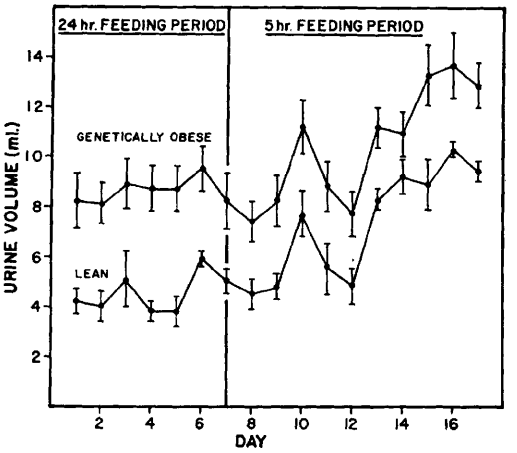

FIG. 1. The effect of a restricted feeding period on urine volumes of lean and genetically obese rats: 14 g of ground chow/day was available to all rats within the periods shown. Urine values of genetically obese rats were significantly greater ($p<0.05$) than those of lean rats on all days except day 14. Vertical bars represent ± standard error of the mean.

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TABLE I. Daily Urine Volume, Body Weight, and Kidney Weight of Rats Maintained on Controlled Food Intake.[a]

	No. of animals	Body wt (g)	Urine vol[b] (ml)		Kidney wt[c] (g)
Lean	6	180.0 ± 4.2	9.2 ± 0.3[d]		1.17 ± 0.10
Lesioned obese	6	203.0 ± 7.5	9.7 ± 0.4	$p < 0.001$	1.59 ± 0.28
Genetically obese	9	219.9 ± 6.3	12.0 ± 0.4		1.35 ± 0.10

[a] Animals were given 14 g of ground chow to eat over a 5-hr period.

[b] Urine volumes were measured daily over a 7-day period.

[c] The animals were sacrificed 4 months after these experiments and the left kidney was weighed. The strict feeding regime was maintained throughout.

[d] Mean ± SE.

period was reduced to only 5 hr so that both groups of animals received their solute load over the same period of time. Under these conditions, urine production increased, but the genetically obese rats still produced significantly greater volumes of urine than the lean control animals. Typical urine volumes of lean, lesioned obese, and genetically obese rats maintained on identical food intakes over a 5-hr feeding period are shown in Table I. All animals ate this food allowance at a similar rate within this time period. Unlike genetically obese animals, the urine production of lesioned obese rats was very similar to that of the lean controls.

The increased urine volume of genetically obese rats was associated with a decrease in urine osmolality and a decrease in urine protein concentration (Table II). Both of these parameters were slightly increased in lesioned obese animals. However, the total osmolal excretion ($U_{osm} \times V$) remained constant in all three groups.

The maximal renal concentrating ability of these rats was investigated by measuring the osmolality of fresh urine samples taken after 24 hr of water deprivation. Both genetically obese and lesioned obese animals were able to concentrate their urine up to the normal values shown by the lean controls (Table III).

The possibility existed that the increased urine production by genetically obese rats resulted from a deficiency in pitressin secretion and/or action. To test this, a supraoptimal dose of pitressin (0.5 U) was injected daily for 5 days. Urine volumes (Table IV) were reduced by a similar amount in all three groups, but still remained significantly larger in the genetically obese rats.

Investigation of water intake over a 4-day period revealed, as expected, a larger water intake in the genetically obese rats (Table V), despite the controlled feeding regime.

The rats were sacrificed at 10 months of age and the kidneys were examined by light

TABLE II. Urine Protein Concentration, Urine Osmolality, and Total Osmolal Excretion of Rats Maintained on Controlled Food Intake.[a,b]

	U_{osm} (mOsm/kg)	p	V_{vol} (ml/24 hr)	p	$U_{osm}V$ (mOsm/24 hr)	Urinary protein (mg/ml)
Lean	2076 ± 67 (9)[c]		8.8 ± 0.5		18.226 ± 1.386	1.64 ± 0.22 (6)
Lesioned obese	2303 ± 219 (9)	< 0.05	8.6 ± 0.9	< 0.01	19.064 ± 1.016	1.83 ± 0.22 (5)
Genetically obese	1658 ± 166 (9)		13.7 ± 1.7		20.722 ± .457	1.31 ± 0.10 (9)

[a] Animals were given 14 g of ground chow to eat over a 5-hr feeding period.

[b] 24-hr urine sample was collected. Osmolality and protein concentration were measured as described in Methods.

[c] Mean ± SE (no. of observations in parentheses).

TABLE III. Maximum Concentrating Power of Rat Kidneys.[a]

	Urinary Osmolality (mOsm/liter)	p
Lean	2674 ± 80.7 (5)[b]	>0.1
Lesioned obese	2672 ± 81.9 (5)	
Genetic obese	2489 ± 62.4 (5)	

[a] Urine was collected under mineral oil over a 3-hr period following 24 hr of water deprivation. Food was available in the normal 5-hr feeding period.

[b] Mean ± SE (no. of observations in parentheses).

microscopy. A small increase in kidney weight was obvious in both obese groups (Table I). However, light microscopy did not reveal any changes in kidney morphology. No evidence for the occurrence of eosinophilic deposits in the glomeruli or tubules could be found.

Discussion. The results clearly demonstrate that genetically obese rats produce more urine than lean or lesioned obese rats, despite a controlled food intake and feeding period. High urine volumes have previously been reported in New Zealand obese (NZO) mice (3), but it is not clear from the report whether their food intake was controlled. Stevenson (4) showed that the ratio of water to food intake in lesioned obese rats fed *ad libitum* was decreased. This was not evident in the present study; indeed, the water balance of lean and lesioned obese rats was very similar.

The increased urine volume of genetically obese rats was not related either to food intake or to the rate at which the food was eaten. Furthermore, the tests of kidney function and renal histology would suggest that there was no impairment in the kidney which could account for the increased urine production. Glomerular lesions and hyalinization have been observed in old obese hyperglycemic mice (5, 6) but no evidence for such lesions was found in the younger rats employed in this study. Although the glomerular filtration rate (GFR) was not measured, any differences in GFR resulting from enlarged kidneys would have favored the lesioned obese rats which had the largest kidneys. However, the excretion of urine by these rats was not different from the lean animals, suggesting that increased kidney weight is unlikely to account for the differences in urine volume.

It was possible that either defective secretion of pitressin or resistance to its action could account for the differences in urine volumes between genetically obese rats and their controls. However, these possibilities seem unlikely since a supraoptimal dose of pitressin did not eliminate the differences.

The increased urine volumes of genetically obese rats can thus only be accounted for by their increased water intake. This suggests that these rats have a genetically inherent greater thirst than lean or lesioned obese rats. However, the possibility that a very low spontaneous rate of secretion of pitressin could lead to increased water intake by habituation cannot be overlooked. Such habituation may not have been overcome by 5 days of pitressin treatment. Evidence has accumulated that water balance is regulated by the lateral hypothalamus where osmoreceptive cells are located (7). It has previously been shown that genetically obese rats are hypothyroid, most probably due to a lack of TSH secretion (8), have impaired fertility

TABLE IV. Effect of Pitressin on Urine Volume of Lean, Lesioned Obese, and Genetically Obese Rats.[a,b]

	Control	p	Pitressin	p
Lean	9.6 ± 0.5 (15)[c]	<0.001	6.7 ± 0.5 (15)	<0.001
Lesioned obese	10.4 ± 0.7 (15)	<0.01	7.9 ± 0.5 (15)	
Genetically obese	13.7 ± 0.9 (15)	<0.01	10.8 ± 0.6 (15)	

[a] Pitressin (0.5 U) was injected daily for 5 days. Control animals received vehicle only.

[b] Animals were given 14 g of ground chow to eat in a 5-hr period. Water was available *ad lib.*

[c] Mean ± SE (no. of observations in parentheses).

TABLE V. Water Intake in Lean, Lesioned Obese, and Genetically Obese Rats.[a]

	No. of rats	Water intake (ml/day)	p
Lean	6	22.0 ± 1.1[b] (24)	
Lesioned obese	5	22.3 ± 1.5 (20)	< 0.01
Genetically obese	9	28.4 ± 1.1 (36)	

[a] Animals were given 14 g of ground chow to eat over a 5-hr feeding period. Water intake was measured daily for 4 days.

[b] Mean ± SE (no. of observations in parentheses).

(9) and lengthened estrus cycles (S. Saidhuddin, personal communication), are hyperphagic (10) and react abnormally to factors known to alter food intake (unpublished observations). All these parameters are controlled by the hypothalamus or hypothalamic–pituitary system. We, therefore, postulate that the primary genetic defect of the genetically obese rat is located in the hypothalamus.

Summary. Increased urine volume and increased water intake were shown in genetically obese rats. The increased urine volume persisted despite controlled solute intake and pitressin treatment. Maximum urine concentrating ability and urine proteinuria were similar in lean, genetically obese, and lesioned obese rats. No histological lesions could be identified. The results were discussed in the light of a possible hypothalamic defect in genetically obese rats.

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