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Effects of Adrenalectomy on Thyroid Function and Insulin Levels in Obese (ob/ob) Mice (40349)

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The finding that hypophysectomy prevents the development of obesity in the genetically transmitted obese (ob/ob) mouse (1) and in the fatty (Zucker) rat (2) has focused attention on possible abnormalities of the hypothalamic–endocrine systems of these animals (3–7). Detailed evaluation of the pituitary-thyroid system has shown no significant abnormalities (8). The reproductive system, however, is immature and pituitary-gonadal feedback is abnormal (9–11). The pituitary-adrenal axis may also be impaired. The adrenal glands are larger (12, 13) and circulating concentrations of corticosterone are higher in ob/ob mice (14, 15). Adrenalectomy reduces the accelerated weight of the ob/ob mouse (16, 17) and improves glucose tolerance (16). Whether these effects are due primarily to adrenalectomy or to the associated reduction in food intake is not known since no pair-gained control mice were used. The present paper reports the effects on body weight, glucose, and insulin concentrations of adrenalectomy in ob/ob mice with that in pair-gained control mice.

Methods and materials. Animals. The 42 lean and 41 obese (C57B1/6J-ob) mice used in these experiments were purchased from the Jackson Laboratories, Bar Harbor, Maine. The lean animals included both heterozygotes (+/ob) and homozygotes (+/+). They were fed Purina Laboratory Chow (Ralston Purina Company, St. Louis, MI).

Experimental procedures. Experiment 1. Fourteen lean and 12 obese animals were bled at 14–15 weeks of age and adrenalectomized 10 days later through a flank incision under ether anesthesia. The experiment was terminated after 34 days. Following adrenalectomy, animals were maintained on 10 μg/day of hydrocortisone sodium succinate and 1% sodium chloride in their drinking water.

Experiment 2. Twenty-eight lean and 29 obese animals were adrenalectomized at 5–6 weeks of age. Hydrocortisone was only used during the early postoperative period. From the third day onward, adrenalectomized animals received 1% sodium chloride as their drinking water but no corticosteroids. Animals were maintained at 25 $\pm$ 1° with a 12-hr cycle of light and dark.

One group with sham-operated obese animals were pair-gained to the adrenalectomized obese animals and another group allowed to eat ad libitum. Pair-gaining was accomplished by giving each mouse 2.2 g/day food and with extra food added or withheld to adjust slightly upwards or downwards for differences in body weight. Blood samples were obtained from the retro-orbital sinus. Animals were fasted for 4 hr prior to sacrifice in exp. 1. In exp. 2 they were bled twice, initially after an overnight fast and 34 days later after a 4-hr fast which followed 1 hr of access to food following an overnight fast. Radioactive ^{131}I (2 μCi) was given 4½ hr prior to sacrifice. Blood was obtained at autopsy and the thyroid, liver, stomach, and salivary glands were removed, weighed and radioactivity assayed by placing tissues in glass tubes and then into a well type scintillation counter. Insulin was assayed by a double antibody radioimmunoassay technique (18) using rat insulin as a standard and iodinated pork insulin as the competitive binder. Glucose was measured by the glucose oxidase method. Statistical comparisons used the Student's "t" test for grouped data.

Results. Experiment 1. The 5 month old obese (ob/ob) mice lost weight following adrenalectomy. At the time of sacrifice, the body weights of adrenalectomized obese animals had declined from 47.9 $\pm$ 1.8 g to 38.1 $\pm$ 1.2 g. By matching the weight of a group of obese sham-operated controls to that of the adrenalectomized animals the effects of reduced food intake could be taken into account. The lean animals showed an initial dip in body weight after adrenalectomy but sub-

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sequently regained it. At autopsy the uptake of ^{131}I by the thyroid of the lean adrenalectomized animals was not significantly higher than in the lean sham-operated pair-gained group. In the adrenalectomized ob/ob mice ^{131}I uptake was similar to that in the lean mice. Intact pair-gained ob/ob mice had lower (but not significantly different) uptake of ^{131}I. Radioactivity in the blood as a percent of the injected dose was significantly higher in the obese adrenalectomized mice than in the obese pair-gained controls (Table I). Prior to surgery the insulin concentrations in the obese (ob/ob) mice were 955 ng/ml compared to 6.5 ng/ml for the lean animal. Adrenalectomy and pair-gaining of ob/ob mice reduced the concentration of insulin to levels that were comparable to those of the lean animals prior to surgery.

Experiment 2. The body weight of the sham-operated obese mice which were fed ad lib rose more rapidly than in the sham-operated lean animals (Fig. 1). Adrenalectomy reduced the rate of weight gain in the obese mouse to nearly parallel that of the lean adrenalectomized or sham-operated controls. During the 56 days from adrenalectomy to the time of the first bleeding the lean adrenalectomized animals gained 4.3 ± .6 g (Table II). The adrenalectomized obese mice gained 6 ± 1.1 g which was not significantly greater than the adrenalectomized lean mice. Sham-operated lean animals gained 5.5 ± 0.4 g whereas the sham-operated obese mice gained 19 ± 0.5 g. At the time of the first bleeding, pair-gained obese animals had been fasted overnight. When the radioiodine was given with the initiation of four hours fast (Table II) the uptake in the neck region in vivo of the adrenalectomized lean animals was significantly higher than in the neck of the sham-operated obese animals. When the experiment was repeated 34 days later the pair-gained animals had been fasted overnight but feeding was allowed for 1 hr prior to the injection of ^{131}I. When the animals were allowed to eat *ad libitum* for 1 hr the uptake of radioactive iodine was significantly lower in the pair-fed than in the adrenalectomized animals. Animals with low uptake of ^{131}I had higher urinary iodide excretion than

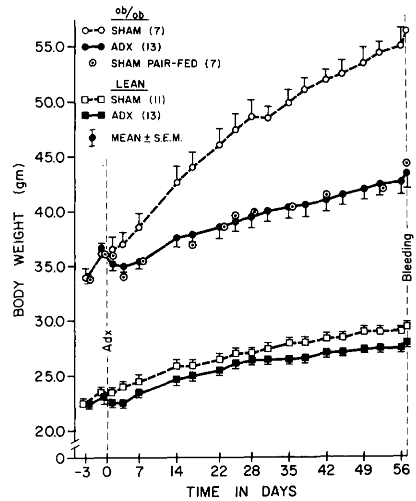

FIG. 1. Weight gain after adrenalectomy. Weight gain over 56 days was measured in sham-operated and adrenalectomized lean or obese mice. A group of sham-operated obese mice were pair-gained to the adrenalectomized animals. The SE of the mean (SEM) is indicated by a line either above or below the point representing the mean.

TABLE I. BODY WEIGHT, THYROID FUNCTION AND INSULIN LEVELS OF OBESE (ob/ob) AND LEAN MICE 6 WEEKS AFTER ADRENALECTOMY OR PAIR-GAINING.

	Lean		ob/ob		
	Sham	ADX	Sham Pair-gained	ADX	P^a
Change in body wt (g)	1.7 ± 0.3[b]	0.2 ± 1.2	−7.3 ± 3.9	−9.8 ± 3.3	<.01
P	N.S.		N.S.		
Thyroid uptake ^{131}I (% of injected dose)	18.9 ± 3.5	23.5 ± 4.8	12.7 ± 3.3	25.1 ± 6.1	N.S.
P	N.S.		N.S.		
Blood radioactivity (% of injected dose)	0.41 ± 0.13	0.49 ± 0.17	0.27 ± 0.10	0.75 ± 0.11	N.S.
P	N.S.		<.01		
Insulin (ng/ml)	2.8	1.0	5.7	4.0	

[a] Comparison of adrenalectomized groups which were 18–19 weeks old at the beginning of the study.
[b] Mean SEM.

animals with higher uptake. The sham-operated obese mice had insulin values that were nearly 50 times higher than the insulin levels of the lean sham-operated animal (Table III). Adrenalectomy reduced the level of insulin in the obese mice as did pair-feeding in sham-operated animals. This suggests that most of the hyperinsulinemia of the ob/ob mouse is secondary to the increased food intake and weight gain in the free-feeding animal. Hyperglycemia of the free-feeding sham-operated obese mouse was significantly higher than in the adrenalectomized or sham-operated pair-fed obese mouse and only slightly higher than that of the lean animals.

Discussion. Phenotypic expression of obesity and hyperphagia in the obese (ob/ob) mouse is profoundly influenced by the pituitary-adrenal axis. The reduction in weight gain and lowered blood glucose has been reported previously (14, 16) but our observations on thyroid function and insulin have not. The inclusion of a control group of ob/ob mice that were fed only enough food to produce changes in body weight similar to those of the adrenalectomized ob/ob mice allows us to distinguish between effects which are attributable to hyperphagia and those due to adrenalectomy. From the two experiments it appears that the reportedly lower uptake of radioactive iodine by the thyroid of the ob/ob mice (8) may result in part from hyperactivity

TABLE II. WEIGHT GAIN AND THYROID FUNCTION OF LEAN AND OBESE (ob/ob) MICE 9 WEEKS AFTER ADRENALECTOMY OR PAIR-GAINING.

	Lean		ob/ob			
	Sham	ADX	Sham	ADX	Sham pair-gained	P^a
Change in body wt (g)	5.5 ± 0.4[b]	4.3 ± 0.6	19.0 ± 0.5	6.0 ± 1.1	8.5 ± 1.5	N.S.
P	N.S.		<.01		N.S.	
Thyroid study I (neck count)	8.4 ± 1.2	25.2 ± 1.4	21.6 ± 2.3	29.6 ± 2.3	30.1 ± 2.5	N.S.
P	<.05		<.05		N.S.	
Blood count (% dose)	0.26 ± 0.02	0.37 ± 0.03	0.31 ± 0.05	0.45 ± 0.05	0.87 ± 0.13	N.S.
P	<.05		N.S.		<.05	
Urine radioactivity (% dose)	65.0	46.0	47.0	30.9	30.7	
Thyroid Study II (% dose)	11.1 ± 0.9	9.9 ± 1.1	7.4 ± 0.7	5.5 ± 0.2	2.9 ± 0.7	<.01
P	N.S.		<.05		<.01	
Blood count (% dose)	0.26 ± 0.01	0.30 ± 0.03	0.31 ± 0.08	0.33 ± 0.05	0.10 ± 0.02	N.S.
P	N.S.		N.S.		<.01	
Urine radiaoctivity	39.5 ± 3.5	46.4 ± 2.2	46.0 ± 7.4	37.4 ± 3.5	70.5 ± 5.3	N.S.
P	N.S.		N.S.		.01	

[a] Comparison of lean and obese adrenalectomized animals which were 5–6 weeks old at the beginning of the experiment.
[b] Mean ± SEM.

TABLE III. INSULIN AND GLUCOSE OF OBESE (ob/ob) AND LEAN MICE NINE WEEKS AFTER ADRENALECTOMY OR PAIR-GAINING.

	Lean		Obese (ob/ob)			
	Sham	ADX	Sham	ADX	Sham Pair-Gained	P^a
Insulin (ng/ml)	4.1 ± 0.9[b]	5.2 ± 1.5	218.0 ± 7.5	81.4 ± 20.3	70.5 ± 11.5	<.01
P	N.S.		<.01		N.S.	
Blood sugar (mg/dl)	50.5 ± 1.5	41.5 ± 0.8	189.5 ± 20.8	88.8 ± 7.9	63.0 ± 11.0	<.01
P	<.01		<.01		N.S.	

[a] Comparison of adrenalectomized lean and obese animals which were 5–6 weeks old at the beginning of the experiment.
[b] Mean ± SEM.

of the adrenal glands with increased losses of iodine in the urine.

The changes in glucose and insulin were almost entirely the result of reduced food intake. Starvation and food restriction are known to restore responsiveness to insulin both *in vivo* (19) and *in vitro* (20). Our findings suggest that the effects of adrenalectomy in restoring glucose and insulin (17) toward normal are the result of reduced food intake. Assimacopolous and Jeanrenaud (4) have argued that many of the metabolic changes in the ob/ob mouse can be explained by the heightened levels of insulin. Thus an explanation of the hyperinsulinemia is central to an understanding of the obese (ob/ob) mouse.

These effects of adrenalectomy on hyperphagia and weight gain in the ob/ob mouse may be explained in two ways. Catecholamines injected directly into the brain can stimulate food intake (21). This effect is markedly reduced after adrenalectomy and is increased over control levels by the injection of corticosteroids (21). Lowering corticosteroids by adrenalectomy might reduce the hyperphagic effects of endogenous brain catecholamines which are known to be increased in concentration in the brain of the ob/ob mouse (22). A second explanation is related to a proposed enzymatic basis for the genetic lesions in the ob/ob mouse (23). It has recently been suggested that a deficiency of the thyroid-inducible component of the sodium transport system in the cell membrane may provide an enzymatic basis for the obesity in these animals (23). The ouabain-inhibitable ($Na^+ + K^+$)-ATPase is involved in the reuptake of catecholamines in the brain, the step which is involved in termination of action. Deficiency of this enzyme at this site might thus enhance the action of catecholamines on feeding.

Summary. The effects of adrenalectomy on the obese mouse were compared using animals which were weight-matched by controlled food intake. Adrenalectomy reduces weight gain of obese (ob/ob) mice. The reduced insulin and glucose after adrenalectomy are largely the result of reduced food intake. Changes in thyroid function are related to both the changes in food intake and to adrenalectomy itself.

1. Herbai, G., Acta Endocrinol. **65,** 712 (1970).
2. Powley, T. L., and Morton, S. A., Amer. J. Physiol. **230,** 982 (1976).
3. Bray, G. A., and York, D. A., Physiol. Rev. **51,** 598 (1971).
4. Assimacopoulos-Jeannet, F., and Jeanrenaud, B., *in* "Clinics in Endocrinology and Metabolism" (M. Albrink, ed.), Vol. 5, p. 337. W. B. Saunders, Philadelphia (1976).
5. Herberg, L., and Coleman, D., Metabolism **26,** 59 (1977).
6. Bray, G. A., Fed. Proc. **36,** 148 (1977).
7. Beloff-Chain, A., Edwardson, J. A., and Hawthorn, J., J. Endocrinol. **65,** 109 (1975).
8. Ohtake, M., Bray, G. A., and Azukizawa, M., Amer. J. Physiol. **233,** R110 (1977).
9. Lane, P. W., Endocrinology **65,** 863 (1959).
10. Swerdloff, R. S., Batt, R. A., and Bray, G. A., Endocrinology **98,** 1359 (1976).
11. Swerdloff, R. S., Peterson, M., Vera, A., Batt, R. A. L., Heber, D., and Bray, G. A., Endocrinology **103,** 542 (1978).
12. Hellerstrom, C., Hellman, B., and Larsson, S., Acta Pathol. Microbiol. Scand. **54,** 365 (1962).
13. Marshall, N. B., Andrus, S. W., and Mayer, J., Amer. J. Physiol. **189,** 343 (1957).
14. Naeser, P., Diabetologia **10,** 449 (1974).
15. Dubuc, P. U., Mobley, P. W., and Mahler, R. J., Horm. Metabol. Res. **7,** 102 (1975).
16. Solomon, J., and Mayer, J., Endocrinology **93,** 510 (1973).
17. Naeser, P., Diabetologia **9,** 376 (1973).
18. Morgan, C. R., and Lazarow, A., Diabetes **12,** 115 (1963).
19. Batt, R., and Mialhe, P., Nature (London) **212,** 289 (1966).
20. Soll, A. H., and Kahn, R. C., J. Biol. Chem. **250,** 4702 (1975).
21. Leibowitz, S. F., 6th Int. Conf. Fd. Water Intake, abstr. (1977).
22. Lorden, J. F., Oltmans, G. A., and Margules, D. L., Brain Res. **117,** 357 (1976).
23. York, D. A., Bray, G. A., and Yukimura, Y., Proc. Nat. Acad. Sci. **75,** 477 (1978).

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