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Isoproterenol Alters Nonshivering Thermogenesis in the Zucker Obese Rat (fafa)[1,2]

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MILAM, K. M., J. S. STERN AND B. A. HORWITZ. *Isoproterenol alters nonshivering thermogenesis in the Zucker obese rat (fafa).* PHARMAC. BIOCHEM. BEHAV. 16(4) 627–630, 1982.—To test nonshivering thermogenic (NST) capacity of lean and obese Zucker rats, 4 doses of isoproterenol, ranging in concentration from 0.25 to 6.0 $\mu g/min \cdot kg^{.75}$, were intravenously infused into 16 to 18 week old male rats, and oxygen consumption was continuously monitored. Obese rats had a decreased NST response relative to lean littermates. This lowered thermogenic response of the obese rats cannot be attributed to a decreased mass of brown adipose tissue since both the cervical and interscapular depots from obese rats weighed significantly more than did those from lean rats.

Obese Zucker rats Brown adipose tissue Isoproterenol-induced thermogenesis

HEAT production is the most direct measure of metabolic rate in mammals. Basal metabolic rate, physical activity and thermogenesis all contribute to the rate of metabolism. Thermogenesis can be broken down into several categories, including diet-induced thermogenesis and thermoregulatory thermogenesis (shivering and catecholamine-stimulated nonshivering), the latter being activated at environmental temperatures below the animal's thermoneutral zone. Since thermogenesis is an effective biochemical mechanism for the dissipation of body energy as heat, its impairment could result in enhanced conversion of dietary energy into carcass energy, i.e., enhanced energetic efficiency. Subnormal nonshivering thermogenesis (NST) has been implicated in the etiology and maintenance of obesity in genetically obese mice (obob) when these mice are maintained below their thermoneutral zone at 24°C [6, 28–31]. Consistent with the occurrence of diminished NST in the obob mouse is the finding that these mice are unable to withstand exposure to cold (4°C) for more than a few hours, apparently due to an inability to sufficiently increase their metabolic rate via nonshivering effectors [6, 23, 28, 29]. Several metabolic insufficiencies, including defects in (Na+/K+)-ATPase [4,33] and reduced binding of purine nucleotides in brown adipose tissue mitochondria [11], may be involved in impaired NST and enhanced energetic storage in the obob mouse.

Evidence for a similar NST deficit in the obese Zucker rat (fafa) is suggestive, but inconclusive. Very young (16 days old) pre-obese Zucker rats can be identified by their lower resting rates of oxygen consumption [9] and rectal temperatures [15] relative to lean littermates. Adult Zucker obese rats also have reduced rates of oxygen consumption when compared to ventromedial hypothalamically-lesioned lean littermates of comparable weight [1]. When exposed to cold temperatures (3–4°C), obese rats initially fail to increase plasma free fatty acid levels [34], rectal temperature begins to drop [34], and death ensues within 28 hours [30]. Diet induced thermogenesis is also reduced in the obese Zucker rat [35]. No metabolic defect has been clearly elucidated to explain these deficiencies in the obese rat although abnormal sympathoadrenal function [18], altered brain catecholamines [5, 16, 17, 18], and/or impaired thyroid function [2, 3, 34, 35] may underlie the disturbed thermoregulation of this obese rodent.

The present study was designed to directly test the capacity of genetically obese rats for nonshivering thermogenesis. Since NST can be effectively stimulated by systemic administration of the β-adrenergic agonist isoproterenol [20], this catecholamine was intravenously infused into lean and obese Zucker rats and its effect on heat production measured.

METHOD

The Zucker rat colony at the University of California, Davis, was the source of lean (Fa/-) and obese (fafa) rats for the present experiment. Homozygous (FaFa) and heterozygous (Fafa) lean rats were included as one group (lean). All rats were individually maintained on Purina laboratory chow in hanging wire cages in a temperature-controlled (24°C) room on a 12-hour light-dark cycle. At 15 weeks of age, the left jugular vein of each rat was cannulated with PE50 tubing (Clay Adams Intramedic Polyethylene Tubing, 7411) under light ether anesthesia. The tubing was passed subcutaneously from the left jugular to the top of the

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TABLE 1

OXYGEN CONSUMPTION (ml O_2/hr·kg$^{.75}$) OF LEAN AND OBESE RATS INFUSED WITH ISOPROTERENOL

Isoproterenol (μg/min·kg$^{.75}$)	Lean (n=5–7) resting	Lean (n=5–7) (ml O_2/hr·kg$^{.75}$) maximal	Lean (n=5–7) Δ*	Lean (n=5–7) %Δ	Obese (n=4–9) resting	Obese (n=4–9) (ml O_2/hr·kg$^{.75}$) maximal	Obese (n=4–9) Δ*	Obese (n=4–9) %Δ
0.25	854.49±25.27	1088.62± 50.22	234.13±43.04	27.4	715.24±24.37	1012.93±41.97	297.70±39.92	41.6
1.0	875.59±23.13	1674.69± 85.25†	799.10±79.68†	91.3	788.78±44.01	963.81±26.77†	193.59±28.54	24.5
3.0	865.65±17.96	1710.32±108.14†	852.08±96.39†	98.4	700.75±11.65	1064.22±22.90†	378.44±16.58	54.0
6.0	859.31±14.56	1635.62± 50.19†	776.31±51.66†	90.3	687.14±12.11	1156.10±52.28†	468.96±46.92	68.2

Values are means±SEM.
*Change in consumption between resting and maximal.
†Lean rats significantly different than obese rats at same isoproterenol dose, $p<0.05$.

skull where it was externalized after being passed through a permanent headmount. The cannula was flushed with a heparin solution (1 mg/ml in saline pH 7.4) every other day.

Oxygen consumption measurements began after a 4 day recovery period. On the morning of the experiment, each rat was put in a Plexiglas restraint. A hole in the top of the restraint was of sufficient diameter to allow the cannula and headmount to pass through, thus providing stability and protection for the cannula. Rates of oxygen consumption were determined on these restrained, unanesthetized rats in a closed system apparatus (Volume Meter, Med-Science Electronics) consisting of a Plexiglas chamber maintained at a constant temperature (24°C) by a surrounding water bath. The experiment began each morning at 8:00 a.m. to minimize circadian fluctuation. Resting levels of oxygen consumption, as defined by Garrow [8], were monitored for 1 hour during infusion of the control vehicle solution (0.1 mg ascorbic acid/ml saline, pH 7.4) at a rate of 4.03 μl/minute. This was followed by a 60 minute infusion of the isoproterenol (0.25, 1.0, 3.0, and 6.0 μg/min·kg$^{.75}$ solubilized in the control vehicle solution), after which the infusate was switched back to the control solution to return the oxygen consumption to resting levels (such a return usually occurred within 1 hour after isoproterenol infusion ceased). Maximal rates of oxygen consumption were determined by averaging the peak oxygen uptake over a 20–30 minute plateau. A single concentration of isoproterenol was used each day, the concentration order going from low to high, with 2 days of recovery between each infusion.

After all rats had been infused with the 4 concentrations of isoproterenol (16–18 weeks of age), they were decapitated at 19 weeks of age. Since brown adipose tissue (BAT) is a major site of catecholamine-stimulated NST in rats [7, 21, 24, 25, 26] cervical and interscapular BAT depots were excised and weighed.

Oxygen consumption data were analyzed using a two-way analysis of variance (ANOVA) test (Table 1). The Newman-Keuls multiple range test was used to analyze differences between means. BAT cellularity and protein determination [19] were both analyzed by Student's t-test; $p<0.05$ was considered significant.

RESULTS

Body weights of the obese rats (500 gm) were significantly heavier than those of the lean rats (334 g), $p<0.05$. Resting rates of oxygen consumption of obese rats (n=11) were significantly less than those of lean rats (n=9) when expressed as a function of body weight (obese: 703.6±7.3 ml O_2/hr·kg$^{.75}$, lean: 864.7±13.5 ml O_2/hr·kg$^{.75}$, $p<0.05$), but not as absolute rates of oxygen uptake (obese: 387.0±5.9 ml/hr lean: 372.9±8.7 ml/hr, $p>0.05$). When oxygen uptake was expressed as a function of body weight, no difference in response to infused isoproterenol at the lowest dose, 0.25 μg/min·kg$^{.75}$, was noted between lean and obese rats (Table 1). However, at the higher doses, lean rats showed a significantly greater maximal response than obese rats ($p<0.05$). A similar effect was noted when the absolute amount of isoproterenol infused (μg/min) was graphed relative to the change in oxygen consumption (expressed as ml O_2/hour) (Fig. 1).

The cervical BAT from lean rats averaged 48.44±3.01 grams compared to 174.98±0.6 grams in the obese ($p<0.05$). By the same token, the interscapular depot from lean rats averaged 282.00±17.10 grams and was significantly less than that found in obese rats, 1220.70±109.60 ($p<0.05$).

DISCUSSION

The heat generating processes which constitute metabolic rate fall into several categories: e.g., basal metabolism, physical activity, diet-induced thermogenesis, and thermoregulatory thermogenesis. Previous work suggest that all four of these heat generating processes may be reduced in obese Zucker rats, thereby contributing to the more efficient conversion of dietary energy into carcass energy stores (i.e., energetic efficiency) in these rats [1, 15, 18, 27, 29, 34, 35].

Although the present study did not measure basal metabolic rates, resting levels of oxygen consumption were measured and, when expressed per unit of metabolic body size, were found to be lower in the obese rats than in the lean (703.6±7.3 vs 864.7±13.5, $p<0.05$, respectively). Similarly, results of the present study substantiate the presence of impaired isoproterenol-stimulated NST (a measure of thermoregulatory heat production) in the obese Zucker rat. Moreover, the fact the decreased thermogenic response of obese rats is more pronounced when oxygen consumption rates are corrected for metabolic mass (i.e, ml O_2·hour·kg$^{.75}$), suggests that the thermogenic capacity of the obese rats is not increased in proportion to their metabolic mass.

Since brown adipose tissue is a major site of NST in rats

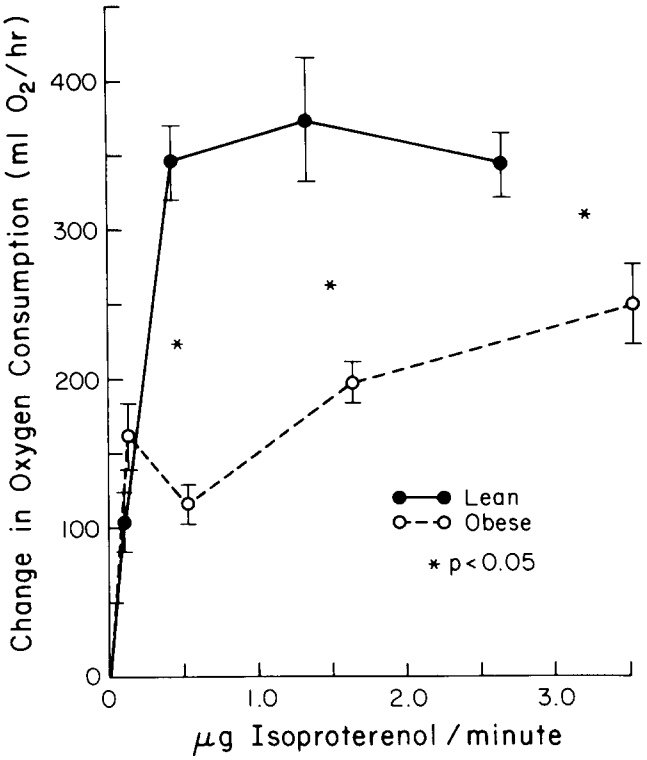

FIG. 1. Effect of infused isoproterenol on oxygen consumption (ml O_2/hr) in lean and obese Zucker rats. Values are means±SEM. *$p<0.05$.

[7, 21, 24–26], the masses of cervical and interscapular BAT from lean and obese rats were compared. The finding that the two brown fat depots in obese rats weighed significantly more than those from lean rats indicates that one cannot attribute the lowered thermogenic responses of the obese rats to a deficit in BAT mass. A cursory examination of tissue samples from the two depots using an osmium fixation technique [12] and an analysis of cell size distribution using a Coulter counter revealed that obese rats had larger cell sizes compared with lean rats. Moreover, in a more detailed study of BAT cellularity in similarly aged male rats that had not been treated with isoproterenol, cervical and interscapular weights and cell sizes were larger in obese than in lean rats [32]. Thus the decreased magnitude of the isoproterenol-induced response in obese rats found in this study may have been due to decreased sensitivity of brown adipocytes to catecholamines rather than any decrease in the amount of BAT. In support of this possibility is the report that norepinephrine-induced oxygen consumption of brown adipocytes isolated from lean Zucker rats was 20 times greater than that of cells isolated from obese littermates [10]. Biochemical factors which may influence the capacity of BAT for thermogenesis in the obese rat include abnormal thyroid metabolism [2, 3, 34, 35], subnormal sympathoadrenal function [18] and/or altered brain catecholamine levels [5, 16, 17]. However, the biochemical lesion causing decreased NST in obese Zucker rats may differ from that in obob mice. Although both obese rats and obob mice have impaired catecholamine-induced NST [28], thyroid function is subnormal in the obese rat [2, 3, 34, 35], but is normal in the obob mouse [22]. Furthermore, activity of (Na^+/K^+)-ATPase, considered essential for normal NST [13,14], is decreased in liver and kidney of obob mice [4,33] but normal in the Zucker obese rat [4]; (Na^+/K^+)-ATPase activity in the thermogenic brown fat has not been determined in obob mice or Zucker obese rats and may differ considerably from liver and kidney activity. Although due to different metabolic anomalies, subnormal thermogenesis appears to contribute to enhance energetic efficiency in both the obob mouse and the obese Zucker rat.

In conclusion, results from the present study confirm the deficit in catecholamine-induced NST in the obese Zucker rat. Moreover, it is clear that this deficiency cannot be explained simply as a decrease in the mass of thermogenic tissue.

REFERENCES

1. Bray, G. A. Oxygen consumption of genetically obese rats. *Experientia* **25:** 1100–1101, 1969.
2. Bray, G. A. and D. A. York. Thyroid function of genetically obese rats. *Endocrinology* **88:** 1095–1099, 1971.
3. Bray, G. A., D. A. York and R. S. Swerloff. Genetic obesity in rats. I. The effects of food restriction on body composition and hypothalamic function. *Metabolism* **22:** 435–442, 1973.
4. Bray, G. A., D. A. York and Y. Yukimura. Activity of (Na^+ + K^+)-ATPase in the liver of animals with experimental obesity. *Life Sci.* **22:** 1637–1642, 1978.
5. Cruce, J. A. F., N. B. Thoa and D. M. Jacobowitz. Catecholamines in the brains of genetically obese rats. *Brain Res.* **101:** 165–170, 1976.
6. Davis, T. R. A. and J. Mayer. Imperfect homeothermia in the hereditary obese-hyperglycemic syndrome of mice. *Am. J. Physiol.* **177:** 222–226, 1954.
7. Foster, D. O. and M. L. Frydman. Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Can. J. Physiol. Pharmac.* **56:** 110–122, 1978.
8. Garrow, J. S. *Energy Balance and Obesity in Man.* New York: American Elsevier Publishing Company, 1974.
9. Godbole, V., D. A. York and D. P. Bloxham. Developmental changes in the fatty (fafa) rat: Evidence for defective thermogenesis preceding the hyperlipogenesis and hyperinsulinemia. *Diabetologia* **15:** 41–44, 1978.
10. Goldberg, J. C. and B. L. G. Morgan. Brown adipose and thermogenesis in lean and obese Zucker rat. *Fedn Proc.* **40:** 871, 1981.
11. Himms-Hagen, J. and M. Desantels. A mitochondrial defect in brown adipose tissue of the obese (ob/ob) mouse: reduced binding of purine nucleotides and a failure to respond to cold by an increase in binding. *Biochem. biophys. Res. Commun.* **83:** 628–634, 1978.
12. Hirsch, J. and E. Gallian. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119, 1968.
13. Horwitz, B. A. Role of active sodium transport in brown fat thermogenesis. *Israel J. med. Sci.* **12:** 1086–1089, 1976.
14. Horwitz, B. A. and M. Eaton. Ouabin-sensitive liver and diaphragm respiration in cold-acclimated hamster. *J. appl. Physiol.* **42:** 150–153, 1977.
15. Kaplan, M. L. Identification of the fa/fa genotype during the pre-obese phase of development. *Fedn Proc.* **36:** 1149, 1977.

16. Levin, B. E. and A. C. Sullivan. Catecholamine synthesizing enzymes in various brain regions of the genetically obese Zucker rats. *Brain Res.* **171:** 560–566, 1979.
17. Levin, B. E. and A. C. Sullivan. Catecholamine levels in discrete brain nuclei of seven month old genetically obese rats. *Pharmac. Biochem. Behav.* **11:** 77–82, 1979.
18. Levin, B. E., J. Triscari and A. C. Sullivan. Abnormal sympatho-adrenal function and plasma catcholamines in obese Zucker rats. *Pharmac. Biochem. Behav.* **13:** 107–113, 1981.
19. Lowry, O., N. Rosebrough, A. Farr and R. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
20. Mejsnar, J. and L. Jansky. Methods for estimation of nonshivering thermogenesis. In: *Nonshivering Thermogenesis*, edited by L. Jansky. Prague: Academia, 1971, pp. 27–38.
21. Nedergaard, J. and O. Lindberg. Norepinephrine-stimulated fatty-acid release and oxygen consumption in isolated hamster brown-fat cells. *Eur. J. Biochem.* **95:** 139–145, 1979.
22. Ohtake, M., G. A. Bray and M. Azukizawa. Studies on hypothermia and thyroid function in the obese (ob/ob) mouse. *Am. J. Physiol.* **233:** R110–R115, 1977.
23. Romsos, D. R., M. J. Hornshuh and G. L. Leveille. Influence of acute thermal stress and maternal diet on metabolic rate of obese (ob/ob) and lean mice at two weeks of age. *Int. J. Obes.* **3:** 249–254, 1979.
24. Rothwell, N. J. and M. J. Stock. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281:** 31–35, 1979.
25. Smith, R. E. and B. A. Horwitz. Brown-fat and thermogenesis. *Physiol. Rev.* **49:** 330–425, 1969.
26. Smith, R. E. and J. C. Roberts. Thermogenesis of brown adipose tissue in cold-acclimated rats. *Am. J. Physiol.* **206:** 143–148, 1964.
27. Stern, J. S. and P. R. Johnson. Spontaneous activity and adipose cellularity in the genetically obese Zucker rat (fafa). *Metabolism* **26:** 371–380, 1977.
28. Thurlby, P. L. and P. Trayhurn. The role of thermoregulatory thermogenesis in the development of obesity in the gentically-obese (ob/ob) mice pair-fed with lean siblings. *Br. J. Nutr.* **42:** 377–385, 1979.
29. Trayhurn, P., P. L. Thurlby and W. P. T. James. A defective response to cold in the obese (obob) mouse and the obese Zucker (fafa) rat. *Proc. Nutr. Soc.* **35:** 133A, 1976.
30. Trayhurn, P., P. L. Thurlby, C. J. H. Woodward and W. P. T. James. Thermoregulation in genetically obese rodents: The relationship to metabolic efficiency. In: *Genetic Models of Obesity in Laboratory Animals*, edited by M. F. W. Festing. In press.
31. Trayhurn, P. and W. P. T. James. Thermoregulation and nonshivering thermogenesis in the genetically obese (obob) mouse. *Pflugers Arch.* **373:** 189–193, 1978.
32. Wickler, S. J., B. A. Horwitz and J. S. Stern. Catecholamine stimulated blood flow to brown adipose tissue is impaired in genetically obese rats (fafa). *Fedn Proc.*, in press, 1982.
33. York, D. A., G. A. Bray and Y. Yukimura. An enzymatic defect in the obese (ob/ob) mouse: Loss of thyroid-induced sodium- and potassium-dependent adenosinetriphosphatase. *Proc. natn. Acad. Sci. U.S.A.* **75:** 477–481, 1978.
34. York, D. A., J. M. Hershman, R. D. Utiger and G. A. Bray. Thyrotropin secretion in genetically obese rats. *Endocrinology* **90:** 67–72, 1972.
35. Young, R. A., O. Tulp and E. S. Horton. Effects of protein malnutrition and thyroid function in Zucker rats. *Clin. Res.* **25:** A305, 1977.