PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 546-548 (1975)

Effect of Force-Feeding Upon Basal Insulin Levels of Rats[1] (39075)

ILENE L. BERNSTEIN, ELIZABETH C. LOTTER, PAUL J. KULKOSKY, DANIEL PORTE, JR., AND STEPHEN C. WOODS

Departments of Psychology and of Medicine, University of Washington and the Seattle Veterans Administration Hospital

A fundamental relationship exists between body weight and basal insulin levels. Obesity is associated with elevated insulin and leanness with reduced insulin (1–6). Further, when a group of humans with a wide range of body weight has been studied within one experiment, a direct correlation with basal insulin has been reported (7–9). This correlation has also been reported for the desert sand rat (10) although it has not been reported for the white rat.

The relationship that exists between adiposity and basal insulin in spontaneously obese or lean subjects could easily be due to discrepancies in diet, environment or exercise. One purpose of the present experiment was to feed rats different amounts of the same diet while maintaining environmental factors constant. Further, since some forms of obesity (11) and hyperinsulinemia (12) in rats are dependent upon a functional parasympathetic nervous system, we also looked at the relation between insulin and adiposity after pretreatment with atropine. We found that basal insulin is directly correlated with body weight of rats and that the relation is unchanged by atropine.

Method. Subjects were twenty female Wistar rats housed in individual stainless steel cages. They were given continuous access to water throughout the experiment and were weighed daily. They were divided into four groups of equal initial average weight. These groups were maintained on one of four feeding regimens. Rats in the Control Group had continuous access to Purina Lab Chow pellets throughout the experiment, and their mean weight was considered the normal weight level for these rats. The other three groups were force-fed different amounts of a nutritionally complete diet, the basic recipe being 350 ml water, 125 g Purina Lab Chow, 100 g Sustagen (Mead-Johnson), 50 g sugar, one cup vegetable oil, and 5 ml formalin (as a preservative), blended to a smooth, thick liquid. Force-feeding was accomplished by gavage; i.e., a polyethylene infant feeding tube was inserted into the mouth, down the esophagus and into the stomach for each feeding. A 20-ml syringe attached to the tube contained a mixture of the diet plus water which was slowly injected intragastrically. The total volume of all injections was increased gradually over the course of the experiment (from 10 to 15 ml), and the proportion of diet to water adjusted such that mean weights of the three groups would differ. Rats in the Obese Group received two (and later three) feedings a day to achieve weights 25% above those of controls. Rats in the Thin Group received two feedings a day to maintain them at 20% below the weight of the controls. Rats in the Average Group received two feedings a day to maintain them at approximately the weight of the controls. Rats in the Obese, Average and Thin groups were also provided with limited access (one pellet/day) to Purina Lab Chow for oral ingestion. Preliminary studies had revealed that the teeth of the force-fed rats grew to excessive lengths and often fractured during insertion of the intragastric tube. Limited access to pellets prevented this problem.

When the rats in the force-fed groups were at the target weights, they were fasted for at least 14 hr and lightly anesthetized with halothane at which time a blood sample (2 ml) was obtained by intracardiac puncture. The procedure was repeated approximately one week later at which time each rat was given an intraperitoneal injection of atropine sulfate (5 mg/kg) ten minutes prior to anesthetization. Immunoreactive insulin (IRI) determinations were subsequently made on the plasma fraction of the blood samples by a previously described double antibody method (13).

Results. Body weight data are summarized

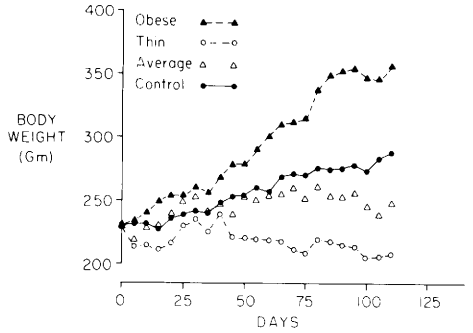

FIG. 1. Mean body weight (g) of the four groups of rats throughout the experiment.

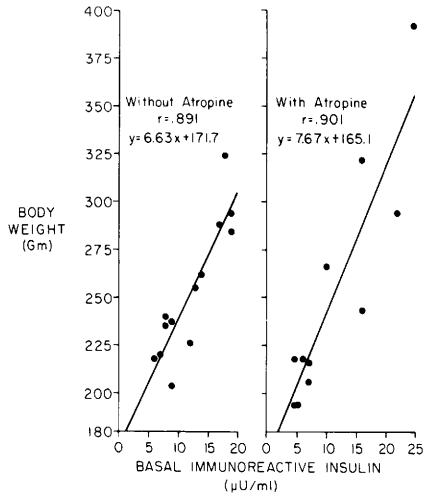

FIG. 2. Body weight (g) and basal immunoreactive insulin levels (IRI: microunits/ml) for the individual force-fed rats in the presence and absence of 5 mg/kg of atropine sulfate.

in Fig. 1. Target weights were achieved after 80–90 days of force-feeding. The Obese Group averaged around 350 g, the Thin Group around 200 g, and the Average Group around 250 g. The mean weights of the Obese and Thin groups were significantly different from those of the Controls at this time.

Plasma IRI, in the absence and presence of atropine, is plotted as a function of body weight for the individual force-fed rats in Fig. 2. Basal IRI levels were directly and highly significantly correlated with body weight in these rats ($r = +.89$). Further, the relationship (as determined by the slope of the regression curves) was essentially unchanged in the presence of atropine ($r = +.90$).

Discussion. Plasma IRI of rats force-fed different amounts of an identical diet were highly, positively correlated with body weight. This finding extends the results of several studies on humans (7–9) and one on sand rats (10) in which dietary factors were not constant. Further, the high positive correlation was virtually unchanged when atropine injections preceded blood sampling. Since this dose of atropine can successfully block a neurally-elicited secretion of insulin by rats (14), the elevated insulin levels observed in the Obese Group were probably not due to an increased neural activation of the pancreas. The hyperinsulinemia of this type of obesity may therefore be more analogous to that of the Zucker rat (15) than the rat with a lesion of the ventromedial hypothalamus (11). The relationship between the nervous system and insulin levels has recently been reviewed (16).

Summary. Rats were over- or under-fed to achieve a wide range of body weights. The effect of this treatment on basal insulin levels, with and without pretreatment with atropine, was examined. Basal insulin was positively correlated with body weight and this relationship was essentially unchanged in the presence of atropine.

This research was supported by Research Grant No. AM 12829 and AM 17112 and from the National Institutes of Health. We thank H. Beiter for his technical assistance. Reprint requests should be addressed to Ilene L. Bernstein, Department of Psychology, NI-25, University of Washington, Seattle, Washington 98195.

1. Woods, S. C., Decke, E., and Vasselli, J. R., Psychol. Rev. **81**, 26 (1974).
2. Yalow, R. S., Glick, S. M., Roth, J., and Berson, S. A., Ann. NY Acad. Sci. **131**, 357 (1965).
3. Beck, P., Koumans, J. H. T., Winterling, C. H., Stein, M. F., Daughaday, W. H., and Kipnis, D. M., J. Lab. Clin. Med. **64**, 654 (1964).
4. Deckert, T., and Hagerup, L., Acta Med. Scand. **182**, 225 (1967).
5. Baig, H. A., and Ezdozien, J. C., Lancet **2**, 662 (1965).
6. Hadden, D. R., and Beif, M. D., Lancet **2**, 589 (1967).
7. Bagdade, J. D., Bierman, E. L., and Porte, D., Jr., J. Clin. Invest. **46**, 1549 (1967).
8. Grant, D. B., Arch. Dis. Child. **42**, 375 (1967).
9. Shima, K., Price, S., and Foa, P. P., Proc. Soc. Exp. Biol. Med. **121**, 55 (1966).

10. Robertson, R. P., Gavareski, D. J., Henderson, J. D., Porte, D., Jr., and Bierman, E. L., J. Clin. Invest. **52,** 1620 (1973).
11. Powley, T. L., and Opsahl, C. A., Amer. J. Physiol. **226,** 25 (1974).
12. Powley, T. L., and Opsahl, C. A., *in* "Hunger: Basic Mechanisms and Clinical Implications" (D. Novin, W. Wyrwicka, and G. A. Bray, eds), New York, Raven Press, in press.
13. Porte, D., Jr., Graber, A. L., Kuzuya, T., and Williams, R. H., J. Clin. Invest. **45,** 228 (1966).
14. Woods, S. C., Amer. J. Physiol. **223,** 1424 (1972).
15. Opsahl, C. A., and Powley, T. .L., Amer. J. Physiol. **226,** 34 (1974).
16. Woods, S. C., and Porte, D., Jr., Physiol. Rev. **54,** 596 (1974).

Received June 6, 1975. P.S.E.B.M. 1975, Vol. 150.