

The influence of epinephrine and fasting on adipose tissue content and release of free fatty acids in obese-hyperglycemic and lean mice

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SUMMARY

Epididymal-fat-pad adipose tissue obtained from obese-hyperglycemic mice exhibited an impairment in its ability to mobilize free fatty acids (FFA) when incubated with epinephrine and in response to a 16-hour fast. Unexpectedly, FFA production by adipose tissue of fed obese mice was significantly greater than that observed in adipose tissue obtained from lean littermates. It is suggested that the impaired mobilization of FFA may be implicated in the etiology of this type of obesity.

Recent investigations have demonstrated several abnormalities in lipogenesis in obese-hyperglycemic mice. Thus, *in vivo* studies revealed an increased incorporation of C¹⁴-labeled acetate into liver and carcass fatty acids even when obese mice were underfed (1, 2). Similarly, liver slices from obese-hyperglycemic mice exhibit an accelerated rate of lipogenesis from acetate *in vitro* (3). In addition, surviving adipose tissue from these obese animals has been reported to have a high rate of lipogenesis when acetate (4) or glucose (5) is used as substrate. Renold *et al.* (6) have recently found that while oxidation of glucose as well as its conversion to fatty acids, glycerol, and glycogen by adipose tissue was about half normal when expressed per mg. of nitrogen; these parameters were in excess of normal in terms of total tissue. Lipogenesis from acetate, on the other hand, was significantly enhanced even when tissue nitrogen was used as a reference standard for calculation (6).

These findings have placed considerable emphasis on accelerated lipogenesis as the factor responsible for the development and maintenance of this form of obesity, while relatively little attention has been paid to the possible contribution of mobilization of lipids from fat depots. On the basis of studies with C¹⁴-labeled palmitic acid incorporated into the diet, Bates *et al.* (7) concluded that fat mobilization is impaired in the obese-hyperglycemic mouse. The present in-

vestigation was designed to explore this facet of the problem by comparing the rate of FFA release from adipose tissue of obese-hyperglycemic mice with that of their nonobese siblings in response to fasting and epinephrine stimulation *in vitro*.

MATERIAL AND METHODS

Obese-hyperglycemic mice in three different phases of obesity and consequently of three age groups and their lean siblings were used. Mice in the static phase of obesity (weight plateaued) were one year old at sacrifice and weighed 58 to 76 g. Their lean controls were in the 29 to 32 g. weight range. The dynamic group (rapid weight gain) of obese mice was 12 to 16 weeks old and weighed 41 to 54 g. in contrast to the lean mice weighing 22 to 30 g. The remaining group was comprised of obese mice in the onset phase of obesity, 5 to 7 weeks old, weighing 28 to 34 g., and their nonobese littermates weighing 18 to 24 g.

Portions (50 to 60 mg.) of epididymal-fat-pad adipose tissue from etherized obese mice and their lean littermates were incubated in a final volume of 1.0 ml. of Krebs-Ringer bicarbonate medium (8) containing 0.1 per cent glucose and 2 per cent bovine serum albumin. Incubation was accomplished at 37°C in an atmosphere of 5 per cent CO₂ and 95 per cent air in a Dubnoff metabolic shaker oscillating at 60 to 70 cycles

per minute. The FFA content of adipose tissue and release into the medium were measured by the method of Dole (9) 3 hours following the addition of epinephrine¹ at a final concentration of 1 μg . per ml. incubation medium or, in the case of the controls, an equal volume of distilled H_2O . Unless otherwise indicated, the mice were subjected to an overnight fast of approximately 16 hours prior to incubation of tissue.

Nitrogen content of adipose tissue was determined by nesslerization (10) on portions of fat-extracted tissue obtained from areas contiguous to fragments incubated for FFA determinations.

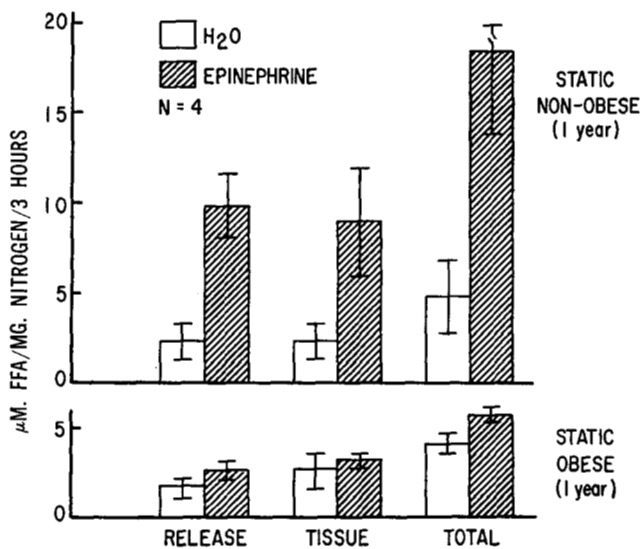


FIG. 1. The influence of epinephrine on adipose tissue content and release of FFA in obese-hyperglycemic mice during static phase of obesity and in their lean littermates.

RESULTS

Static Obesity. Adipose tissue from the fasted, older obese-hyperglycemic mice and from their lean littermates produces approximately equivalent amounts of FFA during incubation without hormonal stimulation. In the nonobese group, epinephrine induces a three- to fourfold increase in the FFA content of tissue, that released to medium, and consequently total production (Fig. 1). Although adipose tissue from the obese-hyperglycemic mice is also influenced by the lipolytic action of epinephrine (Fig. 1), it is relatively insensitive, e.g., the epinephrine effect is only one-ninth that observed for the nonobese group ($p < .01$).

Onset Obesity. The results obtained with adipose

tissue from young obese-hyperglycemic mice, which were gaining weight rapidly, and from their lean littermates, are represented in Figure 2. Again FFA levels in the unstimulated tissues are the same and a lipolytic effect of epinephrine is apparent in both nonobese and obese mice; however, in the latter, the epinephrine effect is significantly diminished ($p < .01$).

Effect of Fasting. The influence of fasting on mobilization of FFA was investigated in a group of obese-hyperglycemic mice in the dynamic phase of obesity and their lean siblings. Fed mice were allowed chow pellets ad libitum. The results are tabulated in Table 1. As expected, nonobese mice show an increase over the baseline release of FFA when fasted ($p < .02$). The total FFA production was of borderline significance ($p = .06$), and probably would be more impressive if more animals were studied. By contrast, the obese mice do not increase the production of FFA to any significant extent during the fast ($p < .1$). However, the rate of fatty acid production by the adipose tissue of the fed obese animals is comparable to that in the fasted lean mice, although the difference between the levels in the fed and fasted obese mice is of questionable significance ($p < .06$). Since this point may be of considerable importance, another series of six lean and obese mice were compared during the fed state. Total production of FFA by the lean mice was $2.1 \pm 0.32 \mu\text{M}$ FFA per mg. N and by the obese $3.6 \pm 0.39 \mu\text{M}$ FFA per mg. N. This difference is significant ($p < .02$). As already observed with fasted onset and static phase obese mice, epinephrine exerts a lesser lipolytic effect on adipose tissue of dynamic

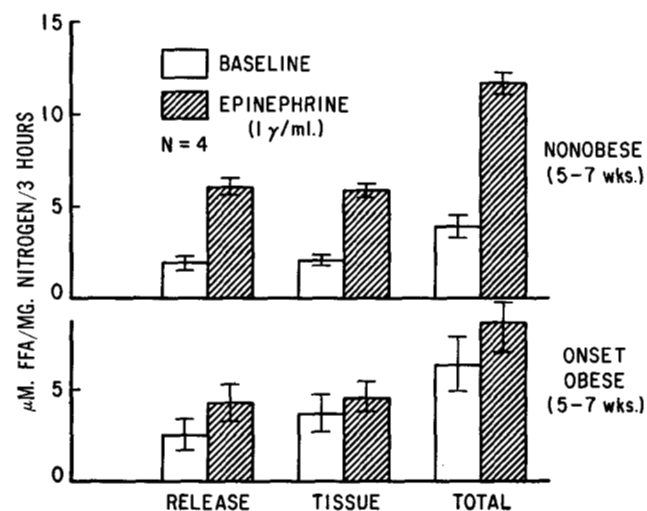


FIG. 2. The influence of epinephrine on adipose tissue content and release of FFA in obese-hyperglycemic mice during onset phase of obesity and in their lean littermates.

¹ Adrenalin®, Parke, Davis and Company, Detroit, Mich.

TABLE 1. THE INFLUENCE OF EPINEPHRINE AND OF FASTING ON ADIPOSE TISSUE CONTENT AND RELEASE OF FFA IN OBESE-HYPERGLYCEMIC MICE DURING THE DYNAMIC PHASE OF OBESITY AND IN THEIR LEAN LITTERMATES

| | No./Grp. | Fed | | Fasted | |
|----------------------------|----------|--|-----------------|----------------|-----------------|
| | | $\mu\text{M FFA}^*/\text{Mg. Tissue Nitrogen}/3 \text{ Hours}$ | | | |
| | | Baseline | Epinephrine | Baseline | Epinephrine |
| Lean Release Tissue | 4 | 1.1 \pm 0.37 | 8.0 \pm 0.95 | 2.9 \pm 0.39 | 6.1 \pm 0.78 |
| | | 1.5 \pm 0.25 | 8.1 \pm 0.46 | 2.0 \pm 0.52 | 7.1 \pm 0.73 |
| | Total | 2.6 \pm 0.62 | 16.1 \pm 0.79 | 4.9 \pm 0.83 | 13.2 \pm 1.37 |
| Obese Release Tissue | 4 | 2.1 \pm 0.29 | 3.4 \pm 0.40 | 2.3 \pm 0.51 | 4.3 \pm 0.47 |
| | | 2.4 \pm 0.33 | 3.4 \pm 0.12 | 2.7 \pm 0.50 | 3.5 \pm 0.24 |
| | Total | 4.5 \pm 0.62 | 6.8 \pm 0.46 | 5.0 \pm 0.58 | 7.8 \pm 0.48 |

* Mean \pm standard error.

phase obese mice both when fed and during fasting than it does in their lean siblings (Table 1).

Nitrogen Content of Adipose Tissue. The data obtained from the nitrogen content of adipose tissue are summarized in Table 2.

TABLE 2. MG. NITROGEN/100 MG. FRESH ADIPOSE TISSUE O-H MICE AND LEAN SIBLINGS

| Age | Nonobese* | Obese* |
|--------------|----------------|----------------|
| 5-7 weeks† | .402 \pm .01 | .130 \pm .01 |
| 12-16 weeks‡ | .255 \pm .01 | .142 \pm .01 |
| 1 Year§ | .198 \pm .01 | .209 \pm .02 |

* Mean \pm standard error.

† Onset obesity.

‡ Dynamic obesity.

§ Static obesity.

During the onset phase of obesity, the nitrogen content of adipose tissue from obese-hyperglycemic mice is only 30 per cent of that found for the nonobese ($p < .001$). As the mice become older, the disparity between obese and nonobese mice tends to disappear, e.g., in the dynamic group, adipose tissue from obese mice contains 80 per cent as much as their lean siblings ($p < .001$). However, during the static phase of obesity, there is no statistically significant differ-

ence between the two groups ($p < .1$). In the obese group, there is no significant difference in adipose tissue nitrogen content between onset and dynamic phases of obesity; however, the difference between static, on the one hand, and onset and dynamic, on the other, is significant at the 1 per cent level. As regards the 5-to-7-week-old nonobese mice, the nitrogen content of adipose tissue is considerably greater than that observed for the two older groups ($p < .001$). The difference between the 12-to-16-week group and the 1-year group is also of statistical significance ($p < .02$).

DISCUSSION

The results of these experiments indicate that in addition to the defects in lipogenesis reported for the obese-hyperglycemic mice, these animals also differ from their normal siblings in their ability to mobilize fatty acids from adipose tissue in response to fasting and epinephrine. In a glucose-containing medium, the lesser net mobilization of FFA by the obese adipose tissue could be due either to a lower rate of lipolysis or to an increased rate of reesterification or to both. Unexpectedly, FFA production from adipose tissue of fed mice in the dynamic phase of obesity was slightly, but significantly, greater than in the controls, being comparable to that of lean mice at the end of a 16-hour fast. Fatty acid production was depressed in adipose tissue from fed lean mice. Perlman *et al.* (11) have recently reported that the FFA content of adipose tissue increases during prolonged fasting in lean but not in obese-hyperglycemic mice. Our data are in agreement, although a shorter fast was employed. The significance of the slightly greater release of fatty acids in the fed obese animals is uncertain, but the inability of these mice to increase fatty acid production during fasting may be of significance in the etiology of this type of obesity.

The failure of the adipose tissue of mice during any phase in the development of obesity to respond to epinephrine to a degree comparable to that exhibited by the lean controls is even more significant. There is now much evidence to suggest that the catecholamine hormones play a significant role in the tonic control of fatty acid mobilization (12, 13). Wertheimer *et al.* (14) have made the interesting observation that Dibenzylamine® blocks the accelerated release of FFA from adipose tissue of rats which have been fasted, exposed to cold, treated with endotoxin, made hyperthyroid with triiodothyronine, or are diabetic. The impaired lipolytic response of the obese mice to epinephrine may thus represent a major defect in the energy

economy of this animal. The inability of the obese-hyperglycemic mouse to withstand hypothermia and to increase its oxygen consumption when exposed to cold (15) might be another reflection of this defect. Finally, it might be noted that Bogdonoff² has recently found that certain obese patients have an impaired plasma FFA response to norepinephrine infusion.

Because of the difference in the thickness and consistency of the fat pads of the obese mice compared to their lean siblings, it might be argued that the diminished lipolytic response to epinephrine is due to a relative inability of the hormone to penetrate the tissue of the obese mice. The difference in response of adipose tissue to fasting in the obese and lean mice would seem to minimize this factor.

The decreasing difference in adipose tissue nitrogen content between obese and nonobese mice with increasing age presumably appears to be related not so much to a decrease in percentage of fat content of obese adipose tissue as to the increase in fat content of nonobese adipose tissue. Apparently the fat content of adipose tissue in the lean group increases with age, while at the same time it is possible that there is a hypertrophy or hyperplasia of adipose tissue, or both, with increasing age in the obese group.

² Personal communication from M. D. Bogdonoff.

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