J. MAYER
R. J. BARRNETT

Department of Nutrition, Harvard School of Public Health and Department of Anatomy, Harvard Medical School, Boston, Massachusetts

SENSITIVITY TO COLD IN THE HEREDITARY OBESE-HYPERGLYCEMIC SYNDROME OF MICE*

The metabolic characteristics of a new Mendelian recessive syndrome in mice have been described in previous publications. The condition is characterized by an adult body weight of 50-100 g. instead of a normal 20-30 g., sterility,[10] hyperglycemia and glycosuria,[17] atrophic changes and ulceration of the skin, and decreased life span. Histopathological studies reveal enlarged islets of Langerhans; no definite histological evidence of other types of endocrine disturbances has been noted.[8] Total basal oxygen consumption is not greater than that of the non-obese animals in spite of a much greater body weight.[19] Spontaneous running activity is low.[15] Although obese animals show sensitivity to thyroxine as indicated by respiratory rate, weight loss, and toxicity, studies on radio-iodine uptake and of resistance to anoxia yield no evidence of impairment of thyroid function.[7] Administration of thiouracil to non-obese animals fails to reproduce any aspect of the syndrome.[20] The caloric intake of the obese mice is about 25 per cent greater than that of the non-obese. If allowed to select their nutrients freely, they choose more fat and less carbohydrate than their normal weight siblings.[18] Hyperglycemia develops at about the twelfth week of postnatal life. This diabetic-like condition is extremely resistant to insulin, is sensitive to growth hormone, and is dependent on the nature and amount of the diet. The obese animals are fatally affected by pancreatectomy and ACTH treatment.[20] They exhibit a partial block of acetate oxidation. By means of tracer techniques, it has been demonstrated in the mice that unoxidized acetate is converted for the main part into fatty acids, in smaller proportion into cholesterol.[8] It has been suggested that there is an etiological relationship between the partial block of acetate oxidation, obesity and hyperglycemia,[20] and between reduced glucose phosphorylation and hyperphagia.[14] The pri-

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mary block to acetate oxidation appears to sensitize the animals to the diabetogenic action of growth hormone. Reduced glucose utilization is, in the glucostatic concept of regulation of food intake,[14, 16, 25] responsible for increased appetite.

While the apparent extreme metabolic inertia of the adipose tissues of these animals can be overcome by fasting,[20] it appeared of interest to see whether in other conditions in which animals have to call on their reserves, the obese mice would be able to do so. Exposure to cold provided such conditions. It is known that, during exposure to cold, resting metabolism can be increased up to four or five times the basal rate.[5, 6] The effect of exercise was also examined, since increased muscular activity is an important component in the resistance to cold.[11] Because the physiological response to cold is mediated through the thyroid[13, 28] and the adrenals,[14] it appeared of interest to test the effect of thyroxine, thyrotrophic hormone, and ACTH on resistance to cold of obese animals as well as the effects of 2-4 dinitrophenol, a hyperthermic agent.[18] The histological effects of exposure to cold on the adrenals and thyroid were examined in both obese and non-obese animals.

METHODS

Young adult mice four to five months were used in these experiments. The weight of the obese animals was 45 to 55 g., that of the non-obese, 15 to 25 g. All animals had been raised in individual cages and fed Purina Laboratory Chow.

A total of 59 obese and 20 non-obese animals were used in the survival experiments. In addition, six obese and six non-obese mice, exposed to cold but not otherwise treated, were used for histological studies. The non-obese mice were killed at the same time the obese succumbed to the cold stress. Untreated obese and non-obese mice kept at 25° C. were sacrificed for histological studies.

The experimental animals were placed in bare, raised-bottomed cages in a cold room maintained at 3° C. Water and food were allowed *ad libitum* unless otherwise mentioned. Activity measurements of eight mice were conducted with the use of squirrel type cages with 32 cm. diameter wheels. Thyroxine, ACTH (Armour), thyrotropic hormone (Armour), and 2-4 dinitrophenol were administered daily by subcutaneous injections during the 48 hours preceding the day of exposure to cold. Dosages are given in Table 2. All animals exposed to cold were observed for survival every 20 minutes during the first two hours, every hour during the next six hours, approximately every two hours afterward until the end of the first day. The total time of survival was noted in each instance.

Adrenal and thyroid glands of the six unexposed obese and non-obese mice, of the untreated obese mice which succumbed in the cold and of their untreated non-obese controls (see above) were weighed and fixed in neutral 10 per cent formalin. Frozen sections at 15μ were made of the adrenals. They were (a) stained with sudan black B or sudan IV, (b) mounted in glycerine and examined with the polarizing microscope,

(c) stained by the Ashbel-Seligman method[2] for carbonyl compounds, or (d) extracted in absolute acetone for one hour at room temperature and treated as in (a) and (b).

The thyroid glands were dehydrated, embedded in paraffin, and sectioned at 5μ in the routine manner. After deparaffinization, the sections were stained with hemotoxylin and eosin and cell heights in the follicles determined according to a method previously described.[22]

RESULTS

Table 1 demonstrates the extreme resistance of non-obese mice to a prolonged cold stress. All exposed animals survived a seven-day exposure

TABLE 1. EFFECT OF SIMPLE EXPOSURE, FASTING, AND ACTIVITY ON THE DURATION OF SURVIVAL IN THE COLD OF HEREDITARILY OBESE-HYPERGLYCEMIC AND CONTROL MICE

	Non-obese mice			*Obese mice*		
Treatment	*None*	*Fasting*	*fasting*	*None*	*Fasting*	*fasting*
Number of animals	12	4	4	21	4	4
Duration of survival (Av. and range)	> 1 week	48 hrs.	15 hrs. (3-48)	2.2 hrs.* (0.5-5.5)	1.5 hrs.	3 hrs. (1-6)
Number of rev./hr.	.....	.....	266 (100-516)	.....	.....	98 (21-112)

* Out of the 21 animals, 19 died between one and three hours after the beginning of exposure.

period. Even when deprived of food, the non-obese mice were still resistant. All exposed animals survived the 48-hour exposure test period. It was only when a combination of fasting and increased activity was used that the limits of resistance to cold of the non-obese mice were overrun. By contrast, obese mice died in about two hours. Fasting and exercise brought about little difference in survival time in the obese mice.

Thyroxine, thyrotrophic hormone, and 2-4 dinitrophenol increased the duration of survival in the cold of exposed obese mice (Table 2). Graded doses of thyroxine had graded effects, but even a large, pharmacological dose of 100 μg. per day (approximately 2 mg./Kg./day) only prolonged survival by a few hours.

On the other hand, corticotrophin was rapidly fatal to the obese animals, definitely shortening their survival in the cold.

Histological examination of adrenals of obese and non-obese animals before exposure to cold showed only slight differences. The zona glomerulosa of non-obese animals contained usually less sudanophilic, birefringent, and Ashbel-Seligman-positive material than the corresponding zone in the obese animals. The lipid droplets of the zona fasciculata exhibited in both obese and non-obese specimens strong reactions to Sudan and the Ashbel-Seligman reagent. The birefringent pattern was somewhat less extensive. The lipid droplets were usually larger and less numerous in the fasciculata of obese mice. The presence of large lipid droplets in this zone is suggestive

TABLE 2: EFFECT OF VARIOUS TREATMENTS ON THE DURATION OF SURVIVAL IN THE COLD OF HEREDITARILY OBESE-HYPERGLYCEMIC MICE

(All treatments administered for 48 hours before exposure)

Treatment	*None*	*Thyroxine (25 μg./day)*	*Thyroxine (100 μg./day)*	*Thyrotrophic hormone (25 mg./day)*	*2-4 dinitro-phenol (25 mg./day)*	*ACTH (25 mg./day)*
Number of obese mice	21	4	4	4	4	4
Average duration of survival and actual values	2.2 (0.5 to 5.5)	3.5 (3, 3, 3, 5)	14.3 (4, 12, 17, 24)	6.5 (4.5, 6.5, 7.8)	3.5 (3, 3, 4, 4)	0.8 (0.3, 0.7, 0.9, 1.3)

of decreased secretory activity. The lack of clear difference in the intensity of staining between the two outer zones in the obese animals was somewhat reminiscent of the pattern characteristic of immature mice. The zona reticularis revealed in the non-obese mice only a few lipid containing cells. There was no difference in the weights of the adrenals of the obese and non-obese mice.

The adrenals of both obese and non-obese animals exposed to cold for only a few hours, including the obese mice which died in this interval, showed no difference from those of unexposed mice. The adrenals of non-obese animals sacrificed after exposure to cold of seven days were enlarged and showed a histochemical pattern typical of the resistance phase of reaction to cold: slight increase in width of the zona fasciculata with cells more heavily laden with small droplets of sudanophilic, birefringent, Ashbel-

Seligman positive material, and, to a lesser extent, increase in positive lipid reactions in the zonae glomerulosa and reticularis.

In agreement with previous observations[8] no difference was noted between thyroid weight of these untreated obese and non-obese animals. In addition, the height of the thyroid follicle cells of obese and non-obese animals kept at room temperature were similar. Exposure to cold for a few hours had no detectable effect on cell height. Non-obese mice after seven-day exposure showed a significant increase in the height of thyroid follicle cells.

DISCUSSION

It is clear from the results presented that mice with the hereditary obese-hyperglycemic syndrome are unable to resist cold stress as successfully as normal animals. Increased sensitivity to cold has previously been observed in mice carrying the "yellow" obese gene.[24] This lack of resistance can, *a priori,* represent either a failure of the mechanisms regulating heat loss or a failure of mechanisms responsible for heat production.

Obese mice have an increased surface area probably not accompanied by a corresponding increase in number of hairs. This may lead to an increase in skin conductivity which may not be counterbalanced by the insulating effect of subcutaneous fat. It was difficult to decide on the basis even of careful observation whether there was any difference in pilo erection or shivering between obese and non-obese mice. No attempt was made to determine whether the obese animals, placed in the cold, were less able than non-obese animals to limit the flow of blood through their skin, tail, paws, and ears. If such an impairment did exist, it would obviously be an important factor.

A number of facts, however, argue in favor of ascribing the sensitivity to cold of obese mice to a failure of additional thermogenesis rather than to a failure of the mechanisms regulating heat loss. Young obese mice, with a near-normal surface area, already show decreased cold resistance. Non-obese mice which carry the "fuzzy" gene and have a very light fur coat display much greater resistance to cold than obese animals.* It appears likely to these authors that mice with the hereditary obesity diabetes syndrome are unable to raise their rate of mobilization and oxidation of reserve metabolites to permit successful resistance to a cold stress. While cold somewhat increases their spontaneous activity as compared to room tem-

* Extremely obese rats with hypothalamic lesions show hardly any decrease in resistance to cold. This syndrome is not characterized by a decrease in ability to burn acetate (report to be published). These findings, incidentally, would argue against Turner's interpretations of the etiology of "yellow obesity."[24]

perature rates,[5] it does not raise it sufficiently to make up for the deficiency in thermogenesis.

Glycogen reserves are low in the obese mice[3, 20] and blood glucose levels are particularly labile in the fasting state.[7] Fasting non-obese mice with depleted glycogen stores still resist cold considerably better than do non-obese mice. The utilization of stored protein does not seem to be implicated.[20] It is known that feeding of protein alone does not improve the resistance to cold of fasted animals.[6] It seems, therefore, highly probable that if there is failure in increasing utilization of reserves, it must be ascribed essentially to impaired mobilization or oxidation of fat. This, furthermore, agrees with the earlier demonstration of a partial block of acetate utilization in obese animals.[8]

While there is abundant proof that the adrenals and the thyroids are intermediaries in the physiological response to the cold stimulus,[1, 11, 12, 23] there is little likelihood that, in the present case, failure to resist cold is due to failure of the response of either the adrenals or the thyroid. Abnormalities of adrenal function have been eliminated as a primary factor in the etiology of this syndrome, since the obesity cannot be reproduced or ameliorated by adrenalectomy, cortical hormone, or ACTH administration.[20] The present studies, furthermore, show that at the time when obese mice die of exposure, the adrenals of non-obese mice do not yet show any morphological or histochemical evidence of increased secretory activity and that ACTH, normally toxic to the obese animals at room temperature,[20] seems also somewhat toxic in the cold. This argues against the possibility that failure of adrenal response alone can account for sensitivity to cold.

Similarly, as mentioned above, abnormalities of thyroid function are not implicated as a cause in the etiology of the syndrome.[3, 15, 19, 20] The extreme metabolic inertia of the adipose tissues has been shown to be responsible for the apparent hypometabolism in the obese mice.[7] While it is true that the weight of the obese animals can be reduced in part (though not to non-obese levels) by administration of large doses of thyroxine, the effect is limited in time. Furthermore, the higher doses of thyroxine required to bring about continued weight loss have a toxic and rapidly lethal effect.[20] In the situation considered here, very large doses of thyroxine, thyrotrophic hormone, and dinitrophenol give some degree of protection to cold, but this effect again is of short duration and appears to be palliative, and not directly curative. It seems likely, therefore, that the same block to fat utilization which is responsible for the obesity is responsible for the sensitivity to cold. While massive doses of thyroxine or allied substances may overcome to some extent or, more likely, circumvent this block, they do not seem to act on the reaction impaired by the primary block.

The results presented here appear to be of significance with respect to an old controversy in the clinical literature. The concept of "lipophilia" introduced by Von Bergmann and Hetenyi[9] implied that the adipose tissue of obese individuals is not mobilized normally. This theory has been denied by many workers. The gist of their arguments, as endorsed and summarized for example by Newburgh,[21] is that if lipophilia existed, obese individuals would not lose weight at the predicted rate when fasted. According to this reasoning, if fat is mobilized by severe fasting, then fat is normally mobilizable. On the other hand, the form of animal obesity studied here illustrates that normal weight loss during caloric restriction[20] may not be incompatible with impaired mobilization of fat under other conditions, such as exposure to cold. It becomes, therefore, questionable whether weight loss under conditions of caloric inadequacy is always an adequate test of the normalcy of fat utilization. For the obese mice to survive either during starvation or during exposure to cold, they have to overcome this block to fat utilization. In starvation, they are capable of doing this, at least for several days. In the cold it may be that they are completely incapable of doing so.*

SUMMARY

1. The hereditary obese-hyperglycemic syndrome of mice causes the animals to be extremely sensitive to cold and to die of exposure in a few hours. This is in contrast to the marked resistance of the non-obese animals, even when these are fasted, or fasted and exercised.

2. Pharmacological doses of thyroxine, thyrotropic hormone, or dinitrophenol prolong significantly, but not considerably, the life of hereditarily obese-hyperglycemic mice exposed to cold. Corticotropin shortens duration of survival under the same conditions.

3. Death occurs in obese animals before non-obese controls show any signs of cortical or thyroid hypertrophy.

4. It appears probable that sensitivity to cold is not due to an impairment of cortical or thyroid function but is due to a primary block in fat utilization. The concept of lipophilia is discussed.

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* Since this article was written, it has been demonstrated (J. Mayer, S. B. Andrus, and D. J. Silides, Endocrinology, in press; J. Mayer, Physiol. Rev., in press) that obese hyperglycemic mice are characterized by a primary hypersecretion of pancreatic hyperglycemic factor, causing a secondary hypersecretion of insulin.

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