Resistance to Hepatic Action of Vasopressin in Genetically Obese (*ob/ob*) Mice

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1. Fatty acid synthesis, measured in the perfused liver of genetically obese (*ob/ob*) mice with $^3\text{H}_2\text{O}$ or [^{14}C]lactate, did not show the inhibition by [8-*arginine*]vasopressin (anti-diuretic hormone) that is observed in livers from normal mice.
2. Hepatic glycogen breakdown in obese mice was stimulated by vasopressin, but not as extensively as in lean mice.
3. If obese mice received a restricted amount of food, then fatty acid synthesis still did not respond to vasopressin, but glycogen breakdown was fully stimulated.
4. Cholesterol synthesis was not inhibited by vasopressin in livers from obese mice.
5. Vasopressin inhibited fatty acid synthesis in intact lean mice, but not in obese animals.
6. These results suggest that genetic obesity could be due to an inborn error within the mechanisms (other than adenylate cyclase) which mediate responses to extracellular effectors.

Genetically obese mice provide a suitable model for the study of the metabolic and endocrine factors that underlie the constitutional type of obesity (for reviews, see Bray & York, 1971; Stauffacher *et al.*, 1971; Loten *et al.*, 1974a). As obesity leads to serious sequelae such as vascular disease and diabetes, this is a major problem in biochemical pathology.

In obese (*ob/ob*) mice, there is excessive synthesis *de novo* of fatty acids, in both liver and adipose tissue (Hems *et al.*, 1975a). Alterations in hormonal concentrations and responses contribute to these increases in fatty acid synthesis. Thus the increased concentration of insulin in blood of *ob/ob* mice (Stauffacher *et al.*, 1967; Abraham *et al.*, 1971), could be implicated (Assimakopoulos-Jeannet *et al.*, 1974; Loten *et al.*, 1974b; Winand *et al.*, 1968). Also, there is resistance to adrenalin action in adipose tissue of *ob/ob* mice, as has been demonstrated *in vitro* by studies of triacylglycerol hydrolysis (Enser, 1970; Yen & Steinmetz, 1972) and adenyl cyclase (Laudat & Pairault, 1975).

Such resistance to hormonal stimulation of fat breakdown could clearly be important in the pathogenesis of obesity. No comparable resistance to hormonal action on lipid metabolism in the liver of obese animals has been reported. In the present paper we report such a property, namely resistance to the inhibition of fatty acid synthesis which vasopressin (anti-diuretic hormone) causes in the liver of normal mice (Ma & Hems, 1975).

Materials and Methods

Mice (female, aged 10–12 weeks) from a random-bred closed colony in the Biochemistry Department, Imperial College, had free access to standard diet (Thompson's formula, provided by Oxoid Ltd.,

London S.E.1, U.K.). Obese mice were homozygous for the *ob* gene, and lean mice were of mixed genotype, i.e. heterozygous, or homozygous for the normal allele. In perfusions of livers of lean mice that were of known genotype, no differences in metabolic rates or responses to vasopressin were detected between the two genotypes. Diet-restricted *ob/ob* mice (weighing about 35 g) were selected at about 6 weeks of age and fed with 4 g at about 10.00 h each day, for 1 month; their eventual steady weight was about 25–35 g.

Livers were perfused between 11:00 and 15:00 h with bicarbonate-buffered medium aged human erythrocytes and glucose and lactate (15 and 10 mM respectively), i.e. in optimum conditions for hepatic lipogenesis (Salmon *et al.*, 1974). Fatty acid and cholesterol synthesis were measured with $^3\text{H}_2\text{O}$ and ^{14}C -labelled precursors and are calculated as C_2 units (i.e. acetyl) incorporated into fatty acids (Ma & Hems, 1975). All perfusions lasted for 3 h, during which the medium was continuously recirculated. [8-*arginine*]Vasopressin (Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.) was added after 45 min, and then at 15 min intervals; radioactive precursors were added after 60 min (Ma & Hems, 1975). Sources of chemicals and analytical methods (for glucose and glycogen) were given previously (Hems *et al.*, 1972).

Experiments in intact mice were carried out as described for rats (Hems *et al.*, 1975b).

Results

Fatty acid synthesis in the perfused liver

In the perfused liver of obese mice, vasopressin at concentrations of 1–10 munits/ml (added repetitively during perfusion) exerted no inhibitory action on fatty acid synthesis (Fig. 1), whereas normal mice

respond to vasopressin concentrations of 1 munit/ml (Fig. 1) or less (Ma & Hems, 1975). Only when sequential doses of 2.5 units were added (so that the concentration added to the perfusate was 50 munits/ml each time), did livers from obese mice

Fig. 1. Influence of vasopressin at various concentrations on fatty acid synthesis

Livers were perfused, in the summer, with $^3\text{H}_2\text{O}$ and ^{14}C lactate. In some perfusions, vasopressin was added in nine sequential doses, each producing the indicated concentration. Fatty acid synthesis was measured between 1 and 3 h: ■, ▲, ●, total synthesized; ○, the amount from lactate. Mice were lean (■), obese fed *ad libitum* (●, ○) or obese on a restricted diet (▲). Other details are in the text (see also Ma & Hems, 1975). Results are means of four perfusions, and bars indicate the S.E.M. One unit of vasopressin is $2.5\text{ }\mu\text{g}$ (about 2.5 nmol).

exhibit a decrease in rate of fatty acid synthesis (Fig. 1). Diet-restricted obese mice also did not respond to vasopressin at repetitive added concentrations of 1 munit/ml (Fig. 1 and Table 1).

Glycogen and glucose metabolism in the perfused liver

In the perfusions described above, glycogen and glucose were measured to assess the response of hepatic glycogen metabolism to vasopressin in obese mice. The output of glucose and the decrease in glycogen content during perfusion with vasopressin were less in livers from obese mice than in lean mice (Fig. 2). These processes were restored to normal by restriction of the amount of food available to obese mice (Fig. 2 and Table 1).

Fatty acid synthesis in diet-restricted obese mice

The results described above suggested that the effect of vasopressin on fatty acid synthesis in obese mice was more irreversibly impaired than the effect on glucose or glycogen metabolism, in that food privation did not restore the normal inhibitory effect of the hormone.

Such irreversibility of the impairment in fatty acid synthesis in obese mice lends greater interest to this characteristic, implying that the lesion may closely reflect their inborn error. Therefore diet-restricted obese mice were investigated further. Perfusions of the liver were carried out during the winter, when the observed rates of fatty acid synthesis in lean mice were faster [a phenomenon that also occurs in the rat (Kirk *et al.*, 1976)]. Again, there was no inhibitory effect of vasopressin on fatty acid synthesis in livers from food-deprived obese mice, whereas glucose output resembled that in livers from lean mice (Table 1). This response (glucose output) confirms that the failure of response of fatty acid synthesis did

Table 1. Fatty acid synthesis and glucose output in perfused livers of diet-restricted obese mice

Livers were perfused during the winter, for 3 h, as described in Figs. 1 and 2. In some groups, vasopressin was added from 45 min, in nine successive doses (each dose as indicated). Fatty acid synthesis was measured with $^3\text{H}_2\text{O}$ and ^{14}C lactate (added after 60 min), and glucose output was calculated from analysis of input and effluent blood at 55 min (i.e. 10 min after vasopressin). Results in lean mice (for comparison, from livers perfused in the winter) are from Ma & Hems (1975). Results are means $\pm$ S.E.M.

Mice	Additions (munits/ml)	No. of perfusions	Fatty acid synthesis (μmol of C_2 units/2h per g)		Glucose output ($\mu\text{mol}/\text{min}$ per g)
			Total	From lactate	
Lean	Control	4	79 ± 3	38 ± 7	0.6 ± 0.2
Lean	Vasopressin (4)	6	37 ± 4	10 ± 2	$4.0 \pm 0.3^*$
Diet-restricted obese	Control	7	46 ± 4	19 ± 2	0.6 ± 0.1
Diet-restricted obese	Vasopressin (1)	3	45 ± 2	18 ± 2	2.1 ± 0.6
Diet-restricted obese	Vasopressin (4)	3	40 ± 8	19 ± 3	3.6 ± 0.4

* Vasopressin 2 munits/ml.

not reflect any inadequacy in the hormone solutions used.

These results showed that in both summer and winter seasons, fatty acid synthesis in livers of obese

Fig. 2. Influence of vasopressin at various concentrations on glucose output and glycogen breakdown

In the perfusions described in Fig. 1, glycogen was measured in the liver at the end of perfusion (a) and glucose output was calculated between 1 and 3 h, from measurement of perfusate glucose (b). Symbols are as defined in Fig. 1.

mice did not respond to vasopressin at concentrations that maximally stimulated glycogen breakdown.

Cholesterol synthesis in the perfused liver

In the perfused livers described above, cholesterol synthesis was also followed, by determining ^3H and ^{14}C in cholesterol. In livers of obese mice, perfused during the summer, there was no inhibition of the conversion of lactate into cholesterol, by vasopressin (Table 2). We have reported such inhibition in livers from lean mice, perfused during the winter (Ma & Hems, 1975; Table 2), in which the total rate of cholesterol synthesis was faster than in the summer. Cholesterol synthesis in diet-restricted obese mice showed no response to vasopressin, at the concentrations tested, in either summer or winter (Table 2).

Responses to vasopressin in intact mice

Experiments with intact mice were carried out to examine the response to vasopressin. The hormone induced an inhibition of fatty acid synthesis in lean mice but not obese mice (Table 3). However, there was immediate hyperglycaemia in response to vasopressin (200 munits) in both lean and obese mice: thus the increase in plasma glucose concentration (7 min after the hormone) was about 4 mM in lean mice and 7 mM in obese mice (results not shown in detail). These results therefore confirmed the findings in the perfused liver. The effects of vasopressin on fatty acid synthesis in obese mice (Table 3) are not statistically significant; any effect on adipose-tissue fatty acid synthesis in particular may be secondary to the increases in plasma glucose and insulin that vasopressin produces (Hems *et al.*, 1975b).

Table 2. Cholesterol synthesis in the perfused liver

Livers of fed female mice were perfused as described in Fig. 1 or Table 1. Cholesterol synthesis was calculated from the ^3H incorporated from $^3\text{H}_2\text{O}$, as described in the text. Results are means $\pm$ S.E.M.; those for lean mice in the winter, for comparison, are from Ma & Hems (1975).

Mice	Additions (munits/ml)	Season	No. of perfusions	Cholesterol synthesized (μmol of C_2 units/2h per g)	
				Total	From lactate
Lean	Control	Summer	4	1.2 ± 0.3	0.5 ± 0.1
Lean	Vasopressin (1)	Summer	4	1.1 ± 0.2	0.4 ± 0.1
Lean	Vasopressin (4)	Summer	3	0.9 ± 0.2	0.3 ± 0.1
Obese	Control	Summer	6	2.1 ± 0.5	0.4 ± 0.2
Obese	Vasopressin (4)	Summer	3	2.1 ± 0.1	0.3 ± 0.1
Obese	Vasopressin (50)	Summer	4	2.8 ± 0.2	0.5 ± 0.1
Diet-restricted obese	Control	Summer	3	0.9 ± 0.1	0.3 ± 0.1
Diet-restricted obese	Vasopressin (1)	Summer	3	0.8 ± 0.1	0.3 ± 0.1
Lean	Control	Winter	5	3.7 ± 0.3	$0.8 \pm 0.2^*$
Lean	Vasopressin (4)	Winter	6	3.1 ± 0.6	$0.3 \pm 0.1^*$
Diet-restricted obese	Control	Winter	7	2.5 ± 0.4	0.8 ± 0.1
Diet-restricted obese	Vasopressin (1)	Winter	3	1.9 ± 0.3	0.7 ± 0.2
Diet-restricted obese	Vasopressin (4)	Winter	3	2.3 ± 0.4	0.7 ± 0.2

* $P < 0.01$.

Table 3. *Effect of vasopressin on fatty acid synthesis in intact mice*

Fed female mice (aged 10–11 weeks) received $^3\text{H}_2\text{O}$ and vasopressin (200 munits) in the summer, intraperitoneally at about 11.00h. After 30 min, liver and parametrial fat were analysed for the ^3H content of fatty acids. Other details are in the text. Results are means $\pm$ S.E.M.

Mice	Treatment	No. of measurements	Total fatty acid synthesis (μmol of C_2 units/g)		$10^5 \times ^{14}\text{C}$ in fatty acid/g (% of injected dose)	
			Liver	Parametrial fat	Liver	Parametrial fat
Lean	Control	6	7.0 ± 1.5	2.7 ± 1.1	7.6 ± 2.6	8.2 ± 4.7
Lean	Vasopressin	7	$3.7 \pm 1.1^*$	2.0 ± 0.4	$1.8 \pm 0.4^*$	9.5 ± 3.9
Obese	Control	4	29.3 ± 1.4	11.5 ± 1.3	9.3 ± 3.5	26.0 ± 10.1
Obese	Vasopressin	4	30.3 ± 3.4	7.1 ± 0.9	27.5 ± 12.8	17.6 ± 5.5

* $P < 0.01$, compared with control value.

Discussion

These results constitute the first evidence in genetically obese mice of a failure of the process of fatty acid synthesis to respond normally to an inhibitory hormone. This impairment is likely to reflect closely the inborn error in these animals, for three reasons: (1) it was not reversible by relatively severe food privation; (2) a lesion in the inhibitory control of fatty acid synthesis would be of crucial pathogenic importance, as obesity is by definition a state in which there is excess deposition of fat; (3) the increase in fatty acid synthesis is intractable to starvation in intact obese mice (Hems *et al.*, 1975a; Volpe & Marasa, 1975; Jansen *et al.*, 1967), as are other processes of lipid metabolism, such as the increased turnover and assimilation of plasma triglyceride (Salmon & Hems, 1973; Rath *et al.*, 1974), free fatty acids and glycerol (Elliott *et al.*, 1974), and the decreased hepatic capacity for ketogenesis (Elliott *et al.*, 1974). Such irreversibility suggests that the lesion in obese mice could reside in the control properties of lipid metabolism.

Therefore the genetic obesity in the *ob/ob* mouse could be a consequence of a defective response in cells, whereby extracellular effectors fail to exert normal constraints, on fatty acid synthesis in particular. Since cholesterol synthesis was also not inhibited by vasopressin [as it is from lactate in particular, in normal mouse liver (Ma & Hems, 1975)], it is possible that the lesion in obese mice tissues is located within the general mechanisms of response of lipid metabolism to effectors, rather than being specific to the pathways of fatty acid synthesis. The observed defect does not involve the adenylate cyclase system, as vasopressin does not act through this system in the liver (Kirk & Hems, 1974; Keppens & de Wulf, 1975).

The above inference is reminiscent of a proposal by Chang *et al.* (1975) based on experiments with lectins, that *ob/ob* mice exhibit a generalized receptor

defect in cells, which does not include impairments of the adenylate cyclase system or of responses to 3':5'-cyclic AMP. However, the defect revealed by the present study cannot be a generalized receptor failure (even of receptor systems other than adenylate cyclase) as glucose output was normally stimulated by vasopressin, in diet-restricted obese mice, at concentrations that act on the liver in normal mice (the present work; Ma & Hems, 1975) or rats (Hems & Whitton, 1973). Also, the defect is not total, as very high concentrations of vasopressin inhibited lipogenesis, even in obese mice fed *ad libitum*. Since the mechanism of vasopressin action on liver metabolism has not been elucidated, the nature of the defect in obese mice cannot be described in specific terms.

The alterations in carbohydrate metabolism in obese mice, which include excessive glycogen synthesis and turnover, and hyperglycaemia, are rapidly and completely reversible by starvation (Abraham *et al.*, 1971; Elliott *et al.*, 1971). The present observation that glucose output by the liver was normally responsive to vasopressin in obese mice that received a restricted quantity of food is in keeping with these earlier findings, in suggesting that changes in hepatic carbohydrate metabolism in obese mice are merely secondary to obesity (Abraham *et al.*, 1971; Elliott *et al.*, 1971). However, the moderate impairment of vasopressin action on glycogen metabolism, in obese mice fed *ad libitum*, may be taken as evidence of a partially defective hormone response, as hepatic phosphorylase activities are increased in obese mice (Das & Hems, 1974). In regard to the stimulation of hepatic glycogen breakdown, or of adenylate cyclase, by glucagon, there appears to be no impairment, even in mice fed *ad libitum*, in *ob/ob* mice (Chang *et al.*, 1975) or in the closely related *db/db* mouse (Chan *et al.*, 1975).

A defect of inhibitory responses in cells in genetically obese rodents (albeit selective in nature) could explain many of the manifestations of these syn-

dromes (reviewed by Bray & York, 1971; Stauffacher *et al.*, 1971; Loten *et al.*, 1974a). In particular, failure of catecholamine action could contribute to their altered adipose-tissue metabolism (Enser, 1970; Laudat & Pairault, 1975), excessive insulin secretion [since catecholamines can inhibit insulin secretion (Mayhew *et al.*, 1969)], and excessive glycogen synthesis in the liver [since adrenalin stimulates glycogen breakdown (Exton *et al.*, 1970; Saitoh & Ui, 1976)]. These considerations again direct attention to short-term actions of extracellular effectors that are not mediated by cyclic AMP (as does the present finding of vasopressin resistance), since at least one major route of adrenalin action in liver is not cyclic AMP-dependent (Sherline *et al.*, 1972; Tolbert *et al.*, 1973; Tolbert & Fain, 1974; Saitoh & Ui, 1976).

In genetically obese mice, the extra lipogenesis occurs in both adipose tissue and liver (Hems *et al.*, 1975a). The present results concern only liver; no impairment of control of fatty acid synthesis in adipose tissue in obese mice has yet been reported. However, there may be a defect in the response of triacylglycerol hydrolysis to adrenaline (Enser, 1970; Yen & Steinmetz, 1972). Also, the impaired adrenalin response of adipose-tissue adenylate cyclase in obese mice (Laudat & Pairault, 1975) may be seen as analogous to the vasopressin resistance reported here for liver, although there was no change in the affinity of the cyclase for the hormone.

There are fewer insulin receptors in the liver of genetically obese rodents, compared with the number in matched lean animals (Kahn *et al.*, 1973; Soll *et al.*, 1975a, b; Chang *et al.*, 1975; Baxter & Lazarus, 1975), although these receptors are functionally normal (Soll *et al.*, 1975b). Also, insulin resistance in the liver, in respect of its actions on carbohydrate metabolism, has been suggested from experiments in intact obese mice (Kreutner *et al.*, 1975). At first sight, the insulin resistance in the liver, inferred in these reports, may seem to resemble the vasopressin resistance described here. However, this characteristic is likely to be a secondary consequence of obesity, as primary insulin resistance would not be expected to cause a predisposition to obesity or to excessive fatty acid synthesis in liver [as occurs in obese mice (Hems *et al.*, 1975a)]. The diminution in the number of insulin receptors in liver of obese mice is indeed reversible by food privation (Chang *et al.*, 1975; Soll *et al.*, 1975a).

It is widely felt that the inborn defect in genetically obese rodents resides at least partly in the hypothalamus. The present work bears on this aspect in two ways. First, the intractability of the defect in the hepatic response to vasopressin (on starvation) suggests that the inborn lesion in the *ob/ob* mice is manifested in non-nervous tissues, such as liver and adipose tissue. Secondly, if the present finding is taken to be representative of the inborn error in

ob/ob mice (as we believe to be warranted), then it follows that their impaired nervous-tissue function will be located in postsynaptic functions (i.e. in responses to transmitters). This remains to be tested.

The particular interest of the vasopressin resistance in the liver of obese mice arises from its persistence in diet-restricted animals. These mice were fed as a routine at 09.00h, whereupon they rapidly consumed their food. Thus their regimen was of a meal-feeding (as opposed to nibbling) type. Livers were perfused at 11.00–13.00h, i.e. soon after feeding. Their blood insulin concentration would then be higher than in lean mice, although lower than in obese mice fed *ad libitum* (Abraham *et al.*, 1971). Such diet-restricted mice have a larger mass of adipose tissue than lean mice of the same weight. Even the intractable vasopressin resistance observed in the present work could perhaps be secondary to an aspect of the meal-fed state, or to excessive insulin action on the liver. Such difficulties have dogged attempts to elucidate the inborn error in genetically obese rodents. For this reason the diet-restricted mice used in the present experiments were severely deprived of food (they received 4g/day), so that they gained no weight over several weeks. The rate of fatty acid synthesis in their livers was lower than that in lean mice (in winter) or in obese mice fed *ad libitum* (in summer), which suggests that insulin action on their liver was not excessive. The return of the full response of glycogen breakdown to vasopressin, after diet restriction, could also carry this implication. Nevertheless no vasopressin response (of lipogenesis) was discerned. Insulin does not prevent the rapid action of vasopressin in the perfused liver of normal mice (G. Y. Ma, unpublished experiments). These considerations indicate that resistance to vasopressin action on the liver could be a direct manifestation of the genetic lesion in *ob/ob* mice, i.e. that the lesion resides within the mechanisms (other than adenylate cyclase) that mediate the responses to extracellular effectors. In the case of vasopressin action on the liver, this mechanism may involve Ca^{2+} ions (M. Stubbs & D. A. Hems, unpublished experiments).

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