CATECHOLAMINES, OBESITY AND INSULIN-RESISTANT DIABETES: EXPERIMENTAL INSIGHTS

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Summary

Recent data from animal models highlight a central role for catecholamines in obesity and insulin-resistant diabetes. Catecholamines have been found to control thermogenic reactions in brown adipose tissue. Obese mutants have low peripheral catecholamine concentrations. Non-obese mutants induced into hyperphagia remain lean by responding with increased amounts of catecholamine-responsive thermogenic brown adipose tissue or remain lean because of genetic abnormalities involving elevated peripheral catecholamine concentrations. Long-term administration of catecholamine analogues prevents the development of obesity, whether from genetic or dietary origins. Finally, the decreased glucose uptake by muscle in obese animals is proposed to result from impaired glucose metabolism; the resistance of the tissue may not be restricted to insulin.

I. INTRODUCTION

Animal models of obesity provide a spectrum of disorders which may be helpful to our understanding of the multifactorial nature of obesity and its frequently associated state of diabetes that occurs in the human. By using these models it has been possible to study the interrelationships between, and the roles played by, genetic background, diet, activity and environment. This paper considers some of the characteristics of genetically obese and diet-induced obese rodents as an insight into the relationship between obesity and insulin-resistant diabetes.

II. OBESITY - GENETICALLY DETERMINED

Most workers regard obesity as simply resulting from an imbalance in the following equation.

energy intake = energy expenditure + energy storage + heat loss

which could reflect either an increase in food intake, an increase in energy storage or a fall in either energy expenditure or heat loss. On this basis it might be expected that the gene defects in the various forms of inherited obesities might be expressed in a protein or groups of proteins which is fundamental to one or more of these parameters.

For the obese Zucker (fa/fa) rat there is evidence that the obese animal consumes more food than its lean sibling during the early stages of development (Bray and York 1972). However, food intake returns towards normal during the static phase of obesity. Hyperphagia appears to occur initially post-weaning. Recent studies using $^{3}H_{2}O$-labelled maternal milk suggest that the fa/fa rat has normal energy intake during weaning despite the storage of excess fat during the pre-weaning period (Boulange et al. 1977). Regardless of hyperphagia it is clear that the hyperphagia itself is not a pre-requisite for the deposition of excess fat in fa/fa rats (Zucker 1975; Bray et al. 1973). Thus a consideration of the energy balance equation shown

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above and the data for the potential variables has led most groups to the notion that many forms of genetic obesity predominantly result from an increase in efficiency of energy utilisation.

The impaired ability of obese mutants to maintain a normal body temperature at laboratory temperature may be indicative of an impaired thermogenic process (van der Kroon 1966; Davis and Mayer 1954; York et al. 1972; Yen et al. 1974; Trayhurn et al. 1977). This thermogenic impairment is not a secondary result of hypothyroidism (Joosten and van der Kroon 1974; York et al. 1978), nor secondary to the obese state, since hypothalamic obesity is not associated with impaired thermogenesis. A low rectal temperature is demonstratable before weaning and before any increase in hepatic lipogenesis or serum insulin (Godbole et al. 1978).

There are many hormonal changes in the ob/ob mouse which are similar to those observed in obese men and women. Nevertheless, many of these changes are probably secondary consequences of the obese state rather than primary defects which determine the difference in metabolic efficiency between lean and obese subjects (James 1976). The importance of non-shivering thermogenesis in obese mice has focused attention on the two hormonal systems most likely to be involved, i.e. the thyroidal and sympathetic catecholaminergic systems (Le Blanc 1975). As indicated above, however, the thyroidal system does not appear to be generally impaired in genetically obese strains. Certainly, in the human, hypothyroidism is rarely found as a cause for obesity, and circulating T_4 and T_3 levels tend to be high rather than low (Bray et al. 1976).

Recent reports suggest that the peripheral concentrations of catecholamines and turnover of catecholamines may be low in genetically obese animals (Levin et al. 1981). Preliminary information on man suggests that venous norepinephrine levels are low in some obese subjects (James et al. 1979) and that this difference persists despite a variety of stimuli including postural changes, the cooling of the extremities and the administration of oral caffeine - a known catecholamine stimulant.

Within the context of hormone involvement in thermogenesis and changes that might contribute to obesity, recent work from three groups indicates that the development of obesity in genetic strains can be halted by the administration of catecholamine-like lipolytic agents. In 1979, Miller (Table 1) showed the effects of ephedrine on six animal models of obesity and a lean control. The drug was incorporated into the diet and the same diet fed to all models (1 g ephedrine/kg). Food intake, metabolic rate, and body weight and composition were expressed as ratios relative to control animals.

Table 1. Effect of ephedrine on various obese models

		Ratios to control animals without drugs				
		Food intake	Oxygen consumption	Body weight	Carcass protein	Carcass fat
Mice	ob/ob	0.79	1.16	0.80	0.86	0.72
	GTG	0.66	1.13	0.64	0.83	0.29
	MSG	0.82	1.22	0.69	0.91	0.43
	E-D	1.02	1.22	0.82	0.98	0.32
	lean	1.00	1.22	0.95	0.94	0.71
Rats	Zucker	0.55	1.25	0.59	0.88	0.34
	E-D	0.72	1.08	0.81	0.84	0.37

GTG: gold thioglucose; MSG: monosodium glutamate; E-D: the energy-dense diet. This table is based on work by Massoudi (1978) and published by Miller (1979).

As shown in Table 1, all models lost body weight and body fat without increasing food intake and had elevated oxygen consumptions. Recently, similar but more extensive data were obtained with ephedrine (Yen et al. 1981) and the ephedrine-like drug LY79771 (Shaw et al. 1981). It is interesting to note that the body fat was reduced without substantial changes in body protein, and hence the fat: N ratio was reduced. This was in contrast to reducing body weight by food restriction, i.e. the animals became more lean irrespective of their body weights.

III. DIET-INDUCED OBESITY IN NON-OBESE STRAINS

McCance and Widdowson (1956) first showed that manipulation of the quantity of diet given from birth to weaning can produce wide differences in growth and body fat. This can be achieved by reducing the number of pups per litter or by manipulation of the quantity of food consumed after weaning. Overfeeding laboratory animals, however, presents a different set of problems. Food intake may be reduced by restricted feeding, or varied by altering the concentration of nutrients, especially protein, but, although such animals may have different growth rates, their carcass composition remains remarkably constant, depending only upon age. Adult animals voluntarily eat about 586 kJ (140 kcal)/$W^{0.75}$ (W = weight in kg). To produce obesity by overfeeding it has been necessary to feed a varied diet, i.e. the 'cafeteria' system (Rothwell and Stock 1977) or to force-feed. Producing obese animals by increasing the quantity of food consumed has its limitations, but varying the quality of the diet has been more productive. This has been achieved by increasing the fat content of the diet. Such a diet has been variously called the high-fat diet, the high-fat-protein diet and the carbohydrate-free diet, but Miller (1979) considers it more appropriate to call it the energy-dense diet since its effects are primarily a result of this function. In our laboratory as well as others (Miller 1979; Lavau and Susini 1975; Rattigan et al. 1982) the energy-dense diet produces obesity in most strains of laboratory animals examined. However, it does not raise the fat content of the already obese Zucker rat (Miller 1979), nor does it have any effect on the spontaneously hypertensive rat (Table 2).

Table 2. Effect of high-fat low-carbohydrate diet on obesity in normotensive and hypertensive rats

	Normotensive Hooded Wistar				Hypertensive Stroke-prone SMR-SP	
	Gonadal and perirenal fat (g)		Body wt.(g)		Gonadal and perirenal fat	Body wt.(g)
High-carbohydrate diet for 150 d	15.0	3.2	397	19(3)	9.8	359(2)
High-fat diet for 150 d	25.1	2.6	410	18(5)	10.9	336(2)

The composition of the diets was as described by Clark et al. (1982).

It is interesting to note that the genetically hypertensive rats show increased peripheral catecholamine levels (De Champlain et al. 1976; Howe et al. 1979). However, the vulnerability of rats to become obese on these high-fat diets is strain-dependent and, whilst readily occurring in most strains, the Wistar Lewis and S5B strains as well as the wild rat do not develop obesity. A relationship between strain differences in this regard and peripheral catecholamine levels has not been ascertained.

Rothwell and Stock (1979) have used a dietary regimen originally described by Sclafani and Springer (1976), which is now referred to as the 'cafeteria' diet. Rats were offered various palatable food items in addition to normal laboratory stock diet. Each day four new food items were offered and the rats, which normally control their food intake precisely, were induced to overeat. On this diet the animals rapidly became obese but, when palatable food items were removed, and only stock diet was available, the body weight rapidly returned to the level of animals fed stock diet throughout (Rothwell and Stock 1977). It has also been possible to induce obesity by including a calorie-dense drinking water ('soft-drink' option) with stock diet (Kanarek and Hirsch 1977). However it is important to note that the degree of obesity obtained using the 'cafeteria' diet and 'soft-drink' option varies between strains and sometimes between individual animals. This difference for the 'cafeteria' diet was initially ascribed to a failure in some experiments to induce hyperphagia. However, in all experiments where food intake was measured there was evidence for marked hyperphagia, suggesting that differences in weight gain were due to variations in heat production. It is now clear that measurement of energy balance during voluntary over-eating in rats has established the quantitative importance of diet-induced thermogenesis (Rothwell and Stock 1979). Like cold-induced thermogenesis, this form of heat production involves changes in the activity of the sympathetic nervous system and brown adipose tissue, suggesting that this tissue may determine metabolic efficiency and resistance to obesity.

Cold-adapted animals show non-shivering thermogenesis in which increased heat production, not associated with muscular activity, is mediated by the sympathetic nervous system and the animals show a pronounced increase in sensitivity to the thermogenic and lipolytic effects of catecholamines (Himms-Hagen 1976; Girardier and Seydoux 1978). The capacity for non-shivering thermogenesis appears to be related to the presence of brown adipose tissue, which is found in many cold-adapted animals, hibernators and the neonates of many species, including the human (Lindberg 1970). Brown adipose tissue has several interesting properties that support conclusions for an important role in thermogenesis. Firstly, in spite of its small total mass (1-2% of the body weight in the cold-adapted rat), brown adipose tissue can receive as much as 34% of the cardiac output under the influence of catecholamines. Secondly, estimates of heat production suggest that this tissue can produce 100 W/kg, representing 60% of the output from non-shivering thermogenesis when stimulated in the cold-adapted rat by catecholamines (Foster and Frydman 1978). Thirdly, the biochemical process for releasing energy as heat during fatty acid oxidation appears to be largely unique to mitochondria from brown adipose tissue. Protons formed during mitochondrial oxidation are dissipated, together with the inherent energy, into the environment, rather than being conserved for ATP synthesis. A protonophoric mitochondrial protein called thermogenin is believed to play a central role (Nicholls 1979). Normally the process is kept under control by GDP, which binds to thermogenin and inhibits proton movement across the mitochondrial membrane (Nicholls 1979). Norepinephrine is now believed to be able to overcome the inhibition by GDP by

increasing cellular levels of acyl CoA; these molecules open up the thermogenin-mediated proton channel by overcoming the GDP binding. Fourthly, enlargement of the brown adipose tissue deposits appears to correlate with the estimated degree of diet-induced obesity. For example, rats fed the 'cafeteria' diet become less obese than expected on food-intake estimates. In these animals the brown adipose tissue increases in weight as does specific thermogenin and total thermogenin. Despite a clear involvement of catecholamines in the regulation of heat production (energy dissipation) in diet-induced thermogenesis, there are still important questions to be considered. For example, there is no indication that β-adrenergic antagonists (blockers of the lipolytic action of norepinephrine) induce obesity in over-fed animals. Also, at present, it is not clear what mechanisms lead to the growth of brown adipose tissue in those animals that are induced into hyperphagia but do not become fat.

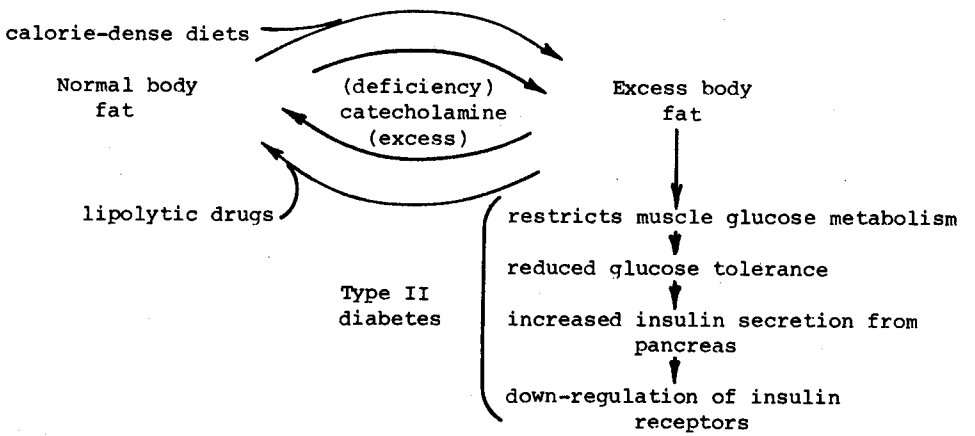
IV. OBESITY AND INSULIN-RESISTANT DIABETES

A complex association between obesity and diabetes may exist. In the human, most patients having symptoms of non-insulin-dependent diabetes are excessively overweight. These same individuals invariably show frequent post-prandial glucosuria, chronic hyperinsulinaemia and a state of insulin resistance illustrated by impaired glucose tolerance and insulin resistance in adipocytes (from biopsy material) and skeletal muscle in vivo (Salans and Cushman 1978). In animal models for obesity, the same variable relationship occurs between obesity and diabetes. Some obese strains of rodents show severe diabetes, insulin resistance and ketosis (e.g. the spiny mouse and the C57 BL/K5-db/db mouse), some show moderate hyperglycaemia and hyperinsulinaemia (e.g. the NZ obese mouse and the KK mouse), and some are normoglycaemic, with moderate hyperinsulinaemia (e.g. the Zucker fa/fa rat) (York 1979). Nevertheless, the simultaneous occurrence of hyperinsulinaemia and normal or elevated blood glucose is suggestive of the insulin-resistant state which develops progressively in all the genetically obese models. For the diet-induced obese rats there is some indication of an accompanying diabetes but more work needs to be done. Generally blood glucose levels are normal in fat-fed obese rats, and insulin levels are elevated (Grundleger and Thenen 1982; Lavau and Susini 1975), but data showing tissue-specific insulin-resistance are sketchy. Impaired basal glucose uptake in adipocytes, diaphragm (Susini and Lavau 1978), soleus muscle (Grundleger and Thenen 1982) and in heart (Rattigan et al. 1982) has been reported for obese fat-fed rats. In addition, the impaired ability of insulin to stimulate glucose uptake in adipocytes, diaphragm and soleus muscle was noted by the same groups of workers. This failure to respond adequately to insulin is referred to as insulin-resistance.

Processes underlying the development of the insulin-resistant state are not fully understood (Olefsky 1976; Czech et al. 1977). The number of insulin receptors on plasma membranes of liver, adipose tissue and muscle of obese animals is decreased (Czech et al. 1977; Olefsky 1976, Soll et al. 1975a, 1975b; Chang et al. 1975; Freychet et al. 1972; Forgue and Freychet 1975). Even though receptor numbers return to normal in obese mutants when serum insulin levels and body weight are reduced, it is not clear that the resistant state arises solely from down-regulation of receptors. Several lines of evidence argue against down-regulation as the primary cause. Firstly, only a small percentage of receptors must be occupied for maximal biological response (Olefsky 1976; Freychet 1976). Secondly, tissue-culture studies show that down-regulation of receptors can be induced by chronic exposure to elevated

insulin levels (Gavin et al. 1974). Thus the relationship between cause and effect becomes clouded. Thirdly, the responses of different metabolic pathways within a single tissue can vary considerably. For example, in ob/ob mice hepatic lipogenesis is maximally stimulated in the hyperinsulinaemic state, whereas the gluconeogenic pathway is totally insensitive to insulin suppression (Assimacopoulos-Jeannet and Jeanrenaud 1976). Fourthly, the basal or unstimulated rate of glucose uptake by tissues from obese diabetic animals is frequently decreased and is often the same proportion below the normal rate as the insulin-stimulated rate is below the corresponding expected insulin-stimulated rate in tissues from normal animals. Fifthly, in some tissues from obese diabetic animals the responsiveness to other hormones that stimulate glucose uptake is impaired. In this regard we have recently shown that glucose uptake by hearts from genetically obese and fat-fed obese rats is markedly diminished and the response to epinephrine, which normally stimulates glucose uptake, is decreased when compared to non-obese control animals (Rattigan et al. 1982). Thus, it appears possible that a defect in the transport of glucose or its intracellular utilisation is an important factor contributing to the insulin-resistant state.

In summary, the recent observations outlined above underscore the central role that catecholamines may play in the development of obesity and insulin-resistant diabetes - at least in animal models. It is clear that norepinephrine controls energy balance by its thermogenic effects on brown adipose tissue. The catecholamines ephedrine and LY 79771 have been successfully used to prevent the development of obesity in genetically obese mutants of rats, mice and beagles and in fat-fed animals. In addition, the ability of rats to gain weight from calorie-dense diets appears to relate inversely to peripheral catecholamine levels, so that Zucker fa/fa rats become obese regardless of calorie density, and spontaneously hypertensive rats that show elevated levels of peripheral catecholamines fail to become obese even on calorie-dense diets. Finally, if the obese state confers an inhibitory effect on glucose uptake it is possible that the diabetic insulin-resistant state that occurs with obesity is a further manifestation of decreased peripheral catecholamine levels or responsiveness. The proposed relationship between catecholamines, obesity and insulin-resistant diabetes is shown below.



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