obese rats. Moreover, the correlation of 3-O-methylglucose with total glucose metabolism indicates that under physiological insulin conditions glucose transport is the rate-limiting step of glucose utilization in hearts of both normal and obese rats.

Basal glycogen synthesis is normal. In contrast, in the presence of insulin, incorporation of glucose is decreased in hearts of obese rats. This indicates that, as in soleus muscle, insulin is unable to bring about a normal stimulation of glycogen synthase.

In heart, it is well established that increased lipid oxidation produces significant increases in concentrations of citrate and subsequently those of glucose 6-phosphate and fructose 6-phosphate (Randle et al., 1966). As no change in the concentrations of these metabolites is observed, alterations of lipid metabolism are unlikely in hearts of obese rats.

The observations that insulin-sensitivity and the maximal insulin effect are not affected in hearts of obese rats indicate that insulin action is not impaired. These hearts are strictly speaking not insulin-resistant. However, both glucose transport and glucose metabolism are decreased by about 50% in hearts of obese rats. These abnormalities may play a role in glucose disposal in vivo and therefore contribute to the state of insulin-resistance of these obese rats.

Sympathetic control of brown adipose tissue in the regulation of body weight

MICHAEL J. STOCK and NANCY J. ROTHWELL
Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17 0RE, U.K.

The influence of the sympathetic nervous system on brown adipose-tissue thermogenesis has been recognized for over 20 years and has received considerable attention from workers interested in NST (non-shivering thermogenesis) (see Himms-Hagen, 1976, for a review). This form of metabolic heat production is seen in many cold-adapted animals, in newborn animals and in hibernators during arousal, but until recently the contribution made by brown adipose tissue to NST was considered small because of its small mass (e.g. less than 1% of body weight in cold-adapted rats). However, Foster & Frydman (1978) demonstrated that the oxygen uptake by brown adipose tissue in the cold-adapted rat in vivo could account for most of NST when activated by noradrenaline, and brown adipose tissue is now recognized as a major thermogenic tissue with an exceptional capacity for oxidation of substrates.

The role of sympathetic activation of this tissue to the regulation of body weight stems from recent studies that have demonstrated that the same mechanisms operate in rats exhibiting DIT (diet-induced thermogenesis). As the term implies, this form of thermogenesis is due to activation of metabolism by food, and it is particularly noticeable when animals are overfed. Miller (1973) has proposed that DIT plays a key role in the regulation of energy balance by allowing for the disposal of energy consumed in excess of requirements. Our studies on this phenomenon have concentrated on using a varied and palatable ‘cafeteria’ diet to induce large (70–80%) increases in the voluntary energy intake of rats, and we have found that this has a potent effect on DIT in many rats, often resulting in a doubling of daily expenditure (Rothwell & Stock, 1979, 1980a).

There are striking resemblances between cafeteria-fed rats exhibiting DIT and cold-adapted rats exhibiting NST, and the high cold-tolerance of cafeteria-fed rats (Rothwell & Stock, 1980c) and the rapid onset of DIT in previously cold-exposed rats (Rothwell et al., 1981a) illustrate how the two thermogenic stimuli produce equivalent metabolic adaptations. The role of the sympathetic nervous system in mediating DIT appears to be similar to that in NST. For example, the high metabolic rates induced by hyperphagia can be inhibited by alpha-adrenergic antagonists, and the thermogenic response to noradrenaline in cafeteria-fed rats is twice that of control animals (Rothwell & Stock, 1979). Ganglionic blockade (with hexamethonium) also abolishes DIT, but alpha-adrenergic antagonists (e.g. phentolamine) are ineffective (Rothwell et al., 1981b).

The enhanced thermogenic capacity of cafeteria-fed rats is associated with pronounced hypertrophy of brown adipose tissue (Rothwell & Stock, 1979), and in young rats there is also evidence of hyperplasia (Tulp et al., 1980). This increase in brown-adipose-tissue adipocyte number is still evident after the cafeteria diet has been withdrawn and can persist into adult life (Brooks et al., 1981). Measurements in vivo (Landsberg et al., 1981) and in vitro (Rothwell et al., 1981b) of noradrenaline release and uptake have provided direct evidence for increased sympathetic activity in brown adipose tissue. Noradrenaline turnover in rats fed on the cafeteria diet for 10 days was increased by 110%, which was similar to the increase (139%) seen in 10-day-cold-adapted rats. All these results indicate a major role for brown adipose tissue in DIT, and recently the quantitative significance of the metabolic changes was established by measurements of blood flow in vivo and oxygen extraction by brown adipose tissue in rats exhibiting high DIT. It was found that the 2-fold increase in thermogenic capacity of cafeteria-fed rats was entirely due to increases in brown-adipose-tissue oxygen utilization (Rothwell & Stock, 1981a).

The increase in brown-adipose-tissue mass of hyperphagic rats is accompanied by changes in histological appearance and biochemical activity similar to those seen in cold-adaptation. Mitochondrial mass and respiratory enzyme activities (e.g. cytochrome oxidase and a-glycerophosphate dehydrogenase) in brown-adipose-tissue depots are increased 2–3-fold, although the specific activities of these enzymes are not altered (Brooks et al., 1980). There is, however, a 2.5-fold increase in the specific GDP-binding capacity of brown-adipose-tissue mitochondria, which, according to Nicholls (1979), indicates increased activity of the proton-conductance pathway.
Scatchard analysis of the GDP-binding characteristics identifies one class of binding site ($K_d = 0.22 \mu M$) and a doubling of $B_{max}$, the maximum number of binding sites (S. L. Brooks, N. J. Rothwell & M. J. Stock, unpublished work), but, in collaborative experiments with Dr. D. Boquier (Université P. & M. Curie, Paris, France), we have been unable to detect significant increases in the 32000-dalton mitochondrial GDP-binding protein involved in the modulation of proton conductance. This suggests that the enhanced GDP binding by brown-adipose-tissue mitochondria from cafeteria-fed rats results from an "unmasking" of existing binding sites after increased sympathetic activation. Evidence for such an effect is presented in Table 1, which shows that injection of rats with noradrenaline 1h before they were killed produces a marked (185%) increase in GDP binding in control rats and an even greater (423%) increase in cafeteria-fed rats. It should be noted that only 3 days of cafeteria feeding are required to produce dramatic changes in mitochondrial GDP binding.

The proton-conductance pathway of brown-adipose-tissue mitochondria is not the only thermogenic mechanism to be implicated in NST or DIT. Horwitz (1979) has suggested that ATP utilization by Na$^+$-K$^+$-stimulated ATPase could provide an alternative or additional means of energy dissipation, and we have subsequently shown a 40% increase in the activity of this enzyme in brown-adipose-tissue homogenates from cafeteria-fed rats (Rothwell et al., 1981b). There was a strong correlation ($r = 0.9$) between the activities of these enzymes and the extent of thermogenesis (resting oxygen consumption) determined several days before animals were killed. The Na$^+$-K$^+$-stimulated ATPase activity of brown-adipose-tissue microsomal fractions exhibited greater sensitivity and maximal responses to additions of noradrenaline in vitro.

The relative importance of proton conductance and ATP utilization by Na$^+$-K$^+$-stimulated ATPase in brown-adipose-tissue thermogenesis is not known, for either NST or DIT. Rothwell (1979) has suggested that ATP utilization by Na$^+$-K$^+$-stimulated ATPase could provide an alternative or additional means of energy dissipation, and we have subsequently shown a 40% increase in the activity of this enzyme in brown-adipose-tissue homogenates from cafeteria-fed rats (Rothwell et al., 1981b). There was a strong correlation ($r = 0.9$) between the activities of these enzymes and the extent of thermogenesis (resting oxygen consumption) determined several days before animals were killed. The Na$^+$-K$^+$-stimulated ATPase activity of brown-adipose-tissue microsomal fractions exhibited greater sensitivity and maximal responses to additions of noradrenaline in vitro.

The relative importance of proton conductance and ATP utilization by Na$^+$-K$^+$-stimulated ATPase in brown-adipose-tissue thermogenesis is not known, for either NST or DIT, but there is little doubt that sympathetic activation of brown-adipose-tissue thermogenesis can exert a major influence on the regulation of energy balance. Apart from the cafeteria-fed rats, perhaps the most obvious demonstration of this influence is seen in genetically obese rodents (e.g. ob/ob and db/db mice). Much of this work has been performed by Trayhurn's group, who have shown that obesity-induced cold-tolerance and exhibit diminished thermogenic responses to noradrenaline (Thurby & Trayhurn, 1979; Trayhurn & James, 1978). Blood-flow studies indicate that the diminished thermogenic responses are due to a failure in brown-adipose-tissue thermogenesis (Thurby & Trayhurn, 1980), which is associated with decreased GDP-binding capacity of brown-adipose-tissue mitochondria (Himmelsbach & Desautels, 1978). In most respects, the obese db/db mouse shows the same defects (Trayhurn, 1979; Trayhurn & Fuller, 1980; Goodbody & Trayhurn, 1981).

The hyperphagic lean rat and the hyperphagic obese mouse illustrate how the regulation of body weight depends on controls operating on metabolic efficiency that can compensate for errors in the control of energy intake. Appetite control has received considerable research attention, and, although a neurophysiological understanding of the mechanisms is still far from complete, the dominant role of the hypothalamus, particularly the ventromedial area, is well recognized. Areas responsible for thermoregulatory control are nearby (e.g. pre-optic area), and it has usually been assumed that this proximity explained the interactions often seen between energy intake and environmental temperature. However, it now appears that the ventromedial area of the hypothalamus is itself involved in the control of heat production. In diabetic rats, acute electrical stimulation of this area activates brown-adipose-tissue thermogenesis via a $\beta$-adrenergic mechanism (Perkins et al., 1981). Electrolytic lesions of the same area have been used extensively to produce obesity in rats, and this has been ascribed to the hyperphagia that follows. However, Seydoux et al. (1981) have now shown that lesions of the ventromedial area of the hypothalamus result in atrophy and inactivation of brown adipose tissue, which would therefore exacerbate the development of obesity. Control of DIT via activation of the ventromedial area of the hypothalamus of brown-adipose-tissue thermogenesis would explain why lesioned animals still become obese when pair-fed together with unlesioned controls.

The afferent signals responsible for influencing hypothalamic control of eating behaviour and thermogenic activity must obviously relate to the influx or amount of nutrients, and insulin has been considered for some time as one of the most important signals of the body's current energy status. Unlike most of the brain, the ventromedial area of the hypothalamus is insulin-sensitive, and several theories of appetite control involving glucoreceptors in the ventromedial area have been proposed. Evidence is now beginning to accumulate that indicates insulin may also act as the hypothalamic signal for activation of DIT. Using diabetic rats maintained on the cafeteria diet, we have recently shown that there is an insulin requirement for DIT, and probably for NST also (Rothwell & Stock, 1981b), and Landsberg et al. (1980) have implicated insulin in the response of the sympathetic nervous system to feeding.

The sequence of events between consumption of food and activation of brown-adipose-tissue thermogenesis is obviously complex, and disruptions at any level could impair an animal's capacity to exhibit DIT, and therefore increase the likelihood of its becoming obese. The defects in ob/ob and db/db mice have been mentioned above, and they seem to indicate that these mutants suffer from a disruption of the effector system, i.e. within brown adipose tissue itself. However, it is possible that the defect lies further back in the regulatory system, and the decreased thermogenic responsiveness and capacity of brown adipose tissue merely reflects atrophy resulting from lack of stimulation. In another genetically obese rodent, the fatty (fa/fa) Zucker rat, we have found some evidence to suggest that the defect is central in origin.

Unlike its murine counterparts, the obese Zucker rat appears to be better equipped for cold-adaptation (Armitage et al., 1981), and we have found that its thermogenic response to injections of noradrenaline is as large as that seen in lean littersmates (N. J. Rothwell, M. E. Saville & M. J. Stock, unpublished work). However, in response to food, the postprandial rise in metabolic rate of the obese rat is less than half that of the lean, and the brown-adipose-tissue temperature response is markedly diminished, or even absent. These preliminary results suggest that the effector pathway for thermogenesis is intact in the obese rat (i.e. normal response to cold and noradrenaline), but signals relating to feeding are absent or ineffective in activating hypothalamic responses, i.e. the animal is physiologically unaware and insensitive to its state of nutrition. Further evidence for this concept comes from the observation that injections of 2-deoxy-D-glucose (which cause

<p>| Table 1. GDP-binding capacity of brown-adipose-tissue isolated from control and cafeteria-fed rats (S. L. Brooks, N. J. Rothwell &amp; M. J. Stock, unpublished work) |
|---|---|---|---|
| Animals received either saline or noradrenaline (25 g/100 g body wt., subcutaneously) 1 h before being killed. Results are mean values ± s.e.m. (n = 6). Effect of cafeteria diet: *$P &lt; 0.05$, **$P &lt; 0.001$. Effect of noradrenaline: †$P &lt; 0.05$, ‡$P &lt; 0.001$. |</p>
<table>
<thead>
<tr>
<th>GDP binding (pmol/mg of protein)</th>
<th>Control</th>
<th>Cafeteria fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-injected</td>
<td>170 ± 41</td>
<td>356 ± 29†</td>
</tr>
<tr>
<td>Noradrenaline-injected</td>
<td>184 ± 45*</td>
<td>1506 ± 150***</td>
</tr>
</tbody>
</table>

1981
glycopenia) caused marked hyperphagia in lean Zucker rats, but were without effect in the obese (Ikeda et al., 1980), and in our experiments injections caused a marked (25%) decrease in metabolic rate in the lean but only a small (8%) decrease in the obese rats. The fact that genetically obese mice exhibit defects in both appetite control and thermogenesis suggests that the genetic lesion in these animals also has a central locus, although it could be argued that defective peripheral mechanisms for thermogenesis could induce the animal to overeat in an attempt to avoid hypothermia.

In this review we have attempted to describe how important sympathetic activity and brown-adipose-tissue thermogenesis are in the regulation of energy metabolism. Many of the mechanisms and concepts are derived from very recent research and have to be thoroughly tested, but there is clearly much scope for future studies in this rapidly advancing field. For us, much of the fascination and excitement in this area lies in the fact that it covers such a wide spread of disciplines, from feeding behaviour, through neurophysiology and endocrinology, to molecular aspects of mitochondrial function. This diversification not only encourages collaboration between workers from many disciplines, but also allows the phenomenon of DIT to be assessed in a broad biological context.


The lactating rat as a model for the study of dietary obesity

DERMOT H. WILLIAMSON, LORANNE AGIUS, MICHAEL R. MUNDAY and ALISON M. ROBINSON
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe, Inffirmary, Woodstock Road, Oxford OX2 6HE, U.K.

The increased mass of adipose tissue in animal models of obesity is usually associated with hyperglycaemia, moderate hyperglycaemia and hyperinsulinaemia (Bray & York, 1979). Lactation in the rat is accompanied by a large increase (up to 300%) in dietary intake, but the increase in body weight is slight (Fell et al., 1963; Cripps & Williams, 1975), and the mass of adipose tissue may actually decrease, depending on the size of the litter (Steinsgrimsdotir et al., 1980). The absence of any appreciable increase in body weight during lactation despite the hyperphagia indicates that the higher intake of energy is balanced by increased expenditure. The site of the increased expenditure of energy is the lactating mammary gland. However, this raises the question of how the available substrates in the circulation, in particular glucose and triacylglycerols, are directed to the gland rather than to adipose tissue for storage. The aim of the present communication is to discuss the possible mechanisms whereby net accretion of lipid in adipose tissue is depressed during lactation. In addition, the value of mammary tissue for studying the regulation of lipogenesis both in vitro and in vivo is briefly considered.

The problem

In the non-lactating rat fed on a normal chow diet (high in carbohydrate), fat deposition in white adipose tissue occurs in three ways: (1) via synthesis of lipid (lipogenesis) de novo within the tissue from lipogenic precursors (mainly glucose), (2) via lipogenesis in liver, secretion of triacylglycerols and transport of these to adipose tissue and (3) uptake of dietary fat in the form of chylomicrons by adipose tissue. Clearly, in the lactating rat the rate of these processes must be altered to provide glucose and triacylglycerols for the formation of milk lipids in the mammary gland. The question is how is this achieved and what are the hormonal signals for the changes?

Triacylglycerols and lipoprotein lipase

The enzyme responsible for the hydrolysis and uptake of triacylgylcerols into adipose tissue is lipoprotein lipase. The activity of this enzyme is high in adipose tissue of non-lactating rats, but it decreases to low values in this tissue at parturition, whereas progesterone acts as a repressor.

Vol. 9


56th MEETING, LANCASTER 527