Insulin-resistance in heart and skeletal muscles of genetically obese Zucker rats

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The obesity syndrome is characterized by hyperinsulinaemia and abnormal glucose tolerance. When normal insulin concentrations can produce only a subnormal effect, a state of insulin-resistance prevails (Kahn, 1978). In obesity, resistance to insulin is usually attributed to an abnormal response of target tissues to the hormone (Crettaz & Jeanraud, 1980). Since heart and total skeletal-muscle mass account for the major proportion of glucose utilization in vivo, studies of metabolic pathways involved in insulin resistance of these tissues are necessary.

Progressive establishment of insulin resistance in muscle

Obesity is a progressive pathology in which insulin-resistance becomes more marked as a function of time (Assimacopoulos-Jeannet & Jeanraud, 1976; Le Marchand et al., 1978). When the insulin effect on glucose metabolism is studied in incubated soleus muscles of obese rats, two phases can be distinguished. First, at 5 weeks of age, a decreased insulin-sensitivity of total glucose metabolism is observed, as higher insulin concentrations are needed to produce the half-maximal effect. At this age, however, the maximal insulin effect (over base-line) is nearly normal. Later, at 10 and 15 weeks, a further decreased insulin-sensitivity is associated with a clear decrease in the insulin effect at supramaximal concentrations of the hormone.

Activation of glucose uptake by insulin increases the rates of glucose incorporation into glycogen and results in increased glucose metabolism concomitantly with the stimulation of glucose transport, glycogen synthase activity and possibly other enzymic steps. Each of these steps may be altered in insulin-resistant tissues.

Main defects in soleus muscle of genetically obese rats

The first step in insulin action is the binding of the hormone to its specific membrane receptor. By yet unknown mechanism(s), this interaction produces in muscle an increase in glucose metabolism concomitantly with the stimulation of glucose transport, glycogen synthase activity and possibly other enzymic steps. Each of these steps may be altered in insulin-resistant tissues.

Insulin binding to soleus muscles of obese rats is characterized by a decrease of 25–35%, which is due to a decrease in insulin-receptor number rather than a change in affinity of insulin for its receptor (Crettaz et al., 1980). This observation may explain the decreased effect of insulin at submaximal concentrations (decreased insulin-sensitivity) of muscles of obese rats. However, since in muscles of normal rats less than 30% of total insulin binding is needed to trigger the maximal effect, a decrease of 25–35% in insulin-receptor number is not sufficient to explain the decrease in the maximal effect observed at supramaximal insulin concentrations.

In muscle, glucose uptake is controlled by glucose transport and phosphorylation. These two steps can be measured by measuring 2-deoxyglucose uptake. Basal uptake by soleus muscles of obese rats is normal, and is decreased by about 50% at 10 weeks of age. Insulin stimulation is clearly impaired at both ages. Since muscle hexokinase activities have been reported to be normal in obese animals (Seidman et al., 1970; Adolfsen et al., 1974), the decrease in 2-deoxyglucose uptake could be due to (still unknown) defect(s) at the level of the hexose-transport system.

Insulin stimulation of glucose into glycogen is markedly diminished in muscles of obese rats when compared with controls. In another model of obesity (the golden hamster induced obese mouse), Le Marchand-Brustel & Freychet (1980) have shown that insulin failed to activate glycogen synthase normally. A similar defect, although not yet demonstrated, could happen and thus explain the discrepancy noted above between the maximal effects of insulin on glycogenolysis and on glucose synthase observed in muscles of 5-week-old obese rats.

Insulin stimulation by increasing the amount of the dephosphorylated active form of the mitochondrial pyruvate dehydrogenase. Chan & Dehaye (1981) have reported that basal and insulin-stimulated concentrations of active pyruvate dehydrogenase were elevated in perfused hindquarters of genetically obese (db/db) mice. The observed decrease in glucose oxidation was interpreted as a decrease in pyruvate availability due to decreased glycogen metabolism. However, end-product inhibition of pyruvate dehydrogenase by changes in acetyl-CoA and NADH concentrations due to increased lipid utilization was not completely ruled out. On the other hand, as glucose and lipids are the main substrates of muscle (Cahill, 1971), it is important to know whether a decrease in glucose metabolism would be compensated for by an increase in lipid oxidation. Therefore lipid utilization is indirectly assessed by measuring O₂ consumption and glucose oxidation. O₂ consumptions are similar in the absence and in the presence of insulin, and are unchanged in muscles of obese rats. Basal and insulin-stimulated glucose oxidation rates are normal in muscles of 5-week-old obese rats, indicating normal lipid utilization. By contrast, at 10 weeks of age, glucose oxidation is decreased, indicating increased lipid oxidation in muscles of obese rats. In keeping with the glucose-fatty acid cycle (Randle et al., 1963), this increase results in elevated citrate and glucose 6-phosphate concentrations measured in absence of insulin. These observations suggest that increased lipid utilization inhibits glycolysis at the level of phosphofructokinase and thus is partly responsible for the decreased glucose metabolism.

Interestingly, glycogen content of muscles of 10-week-old obese rats is elevated, although glycogen synthesis de novo is markedly decreased. According to our observations, a possible explanation is that, in vivo, muscles of obese rats preferentially oxidize lipids and thus spare glycogen stores.

Defects in hearts of genetically obese rats

In order to generalize our observations made on soleus muscle, heart metabolism is studied. Perfused heart is preferred to perfused hindlimb, because contamination by adipose tissue of this latter preparation is not negligible (Ruderman et al., 1971). In resting animals, however, heart contributes a significant proportion in glucose utilization (Neely & Morgan, 1974), and may therefore contribute to the abnormal glucose handling observed in obesity.

Insulin stimulates glucose metabolism of heart and soleus muscle 2–3-fold. However, heart is much less sensitive to insulin than is soleus muscle (insulin concentrations required to produce half-maximal effect are 5–7 munits/ml and 0.05–0.1 munit/ml respectively).

In hearts of 13–15-week-old obese rats basal and insulin-stimulated glucose metabolisms are clearly decreased. It is noteworthy that the absolute decrease measured in the absence of insulin is observed at each insulin concentration tested. Neither insulin-sensitivity nor maximal insulin effect over base-line is altered, and therefore defect(s) distal to the insulin receptor should exist.

Glucose-transport activities are assessed by measuring the efflux of 3-O-methylglucose from hearts preloaded with 3-O-methyl[14C]glucose and i-1[14H]glucose. In the absence of insulin glucose transport is markedly decreased in hearts of...
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

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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, 1980b).

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 adaptions. 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 β-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 α-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 α-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.