REVIEW

A role for suppressed skeletal muscle thermogenesis in pathways from weight fluctuations to the insulin resistance syndrome

A. G. Dulloo
Division of Physiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland

Abstract
An impressive body of epidemiological evidence suggests that a history of large perturbations in body weight earlier in life, independently of excess weight, is a risk factor for later development of insulin-related complications, namely central obesity, type 2 diabetes and cardiovascular disease. Such an increased risk has been reported in men and women who in young adulthood experienced weight fluctuations that involved weight recovery after weight loss caused by disease, famine or voluntary ‘yoyo’ dieting, and is particularly strong when the weight fluctuations occurred much earlier in life and are characterized by catch-up growth after foetal and/or neonatal growth retardation. As the phase of weight recovery/catch-up growth is associated with both hyperinsulinaemia and an accelerated rate for recovering fat mass (i.e. catch-up fat), the questions arise as to whether, why and how processes that regulate catch-up fat might predispose to hyperinsulinaemia and to insulin-related diseases. In addressing these issues, this paper first reviews evidence for the existence of an adipose-specific control of thermogenesis, whose suppression contributes to the phenomenon of catch-up fat during weight recovery/catch-up growth. It subsequently concentrates upon recent findings suggesting that: (i) such suppression of thermogenesis directed at catch-up fat is accompanied by a redistribution of glucose from skeletal muscle to white adipose tissue, and (ii) substrate cycling between de novo lipogenesis and lipid oxidation can operate as a thermogenic effector in skeletal muscle in response to signalling interactions between leptin and insulin – two key ‘adiposity’ hormones implicated in the peripheral control of substrate metabolism. These new findings are integrated into the proposal that, in its ‘evolutionary adaptive’ role to spare glucose for rapid rebuilding of the fat stores, suppressed thermogenesis in skeletal muscle – via inhibition of substrate cycling between de novo lipogenesis and lipid oxidation – confers to the phase of weight recovery/catch-up growth its high sensitivity towards the development of insulin resistance and hyperinsulinaemia, and hence towards diseases that are clustered around the insulin resistance syndrome.

Keywords catch-up growth, low birth weight, obesity, thermogenesis, weight cycling.
Weight fluctuations in children and adults: a risk for later chronic metabolic diseases

Obesity, type 2 diabetes, and hypertension – three major contributors to cardiovascular diseases – have long been recognized to be diseases of civilization. They result from a complex interplay between genetic susceptibility and environmental factors, with insulin resistance (the decreased ability of peripheral target tissues to respond properly to normal concentrations of insulin) as a relatively early development in all three entities (Landsberg 1986, Neel et al. 1998). While it is well established that obesity predisposes to the development of insulin resistance and to type 2 diabetes, hypertension and coronary heart diseases, the analysis of several large epidemiological databases has revealed that large fluctuations in body weight at some point earlier in life (whether during growth or young adulthood) also represent an independent risk factor for the development of type 2 diabetes and cardiovascular diseases.

High cardiovascular morbidity and mortality have indeed been reported in men and women who in young adulthood experienced marked fluctuations in body weight (Hamm et al. 1989, Holbrook et al. 1989, Lissner et al. 1989, 1991, Lee & Paffenbarger 1992). These associations were found to be independent of excess weight and trend of body weight over time, and are hence of clinical relevance not only for the obese ‘yoyo’ dieters who fail to maintain their lost weight, but also for non-obese population groups whose weights fluctuate because of chronic diseases, such as those suffering from chronic alcoholism and gastrointestinal diseases. In some of these cohorts, even a single cycle of substantial weight loss and weight recovery in young adulthood was found to be a risk factor for subsequent coronary heart diseases and mortality (Holbrook et al. 1989, Lissner et al. 1989). Perhaps the most compelling evidence linking large fluctuations in body weight and cardiovascular risks later in life derives from several longitudinal studies which suggest that people who had low birth weight and/or whose growth faltered during infancy and childhood, but who subsequently showed catch-up growth, had higher susceptibility for central obesity, type 2 diabetes and cardiovascular diseases later in life (Cianfarani et al. 1999, Eriksson et al. 1999, Huxley et al. 2000, Ong et al. 2000, Levy-Marchal et al. 2002). For example, the analysis of data from Finland indicates that men who were thin at birth but subsequently showed catch-up growth and became overweight in childhood were found to have a fivefold increase in mortality compared with men with a high body mass index at birth and lean in childhood (Eriksson et al. 1999). Further evidence that catch-up growth, though beneficial in the short term, might be detrimental in the long term can be derived from studies conducted in countries undergoing nutrition transition. Studies from South Africa, Brazil, Russia and China suggest that stunted children have a 2–8 times greater risk of becoming overweight (Popkin et al. 1996) and/or have increased incidence of cardiovascular and metabolic disorders (Levitt et al. 2000, Victora & Barros 2001, Sawaya et al. 2003). Furthermore, in a recent prospective study in Chile, reduced insulin sensitivity could be related to catch-up growth in small-for-gestational age infants at 1 year of age (Soto et al. 2003), while in Switzerland, children born small-for-gestational age were found to have impaired glucose oxidation after glucose ingestion already before puberty (Jornayvaz et al. 2004).

How does such perturbations in body weight lead to the development of high risks for these chronic diseases that cluster around the insulin resistance syndrome (Reaven 2005)? Current hypotheses centre upon the notion that food deprivation or malnutrition, particularly when it occurs during critical periods of growth and development, can lead to lasting alterations in structures and functions of tissues, and in the resetting of major neuroendocrine systems (Hales & Barker 2000, Young 2002). Such malnutrition-induced ‘programming’ or ‘imprinting’, although adaptive during the period of limited supply of nutrients, is thought to contribute to the increased risks for diseases during improved nutrition and weight recovery later in life. Whatever the mechanisms by which programming for such thrifty phenotype predisposes to chronic metabolic diseases, however, a common denominator in many situations of large weight fluctuations – whether during growth or in adulthood – is that body fat is recovered at a disproportionately faster rate than that of lean (muscle) tissue (Table 1), thereby underscoring a potentially pivotal link between processes that lead to accelerated recovery of the fat stores (or catch-up fat) and higher cardiovascular risks. What then is known about processes that regulate catch-up fat after growth retardation or weight loss in adults? An exaggerated increase in energy intake (compensatory hyperphagia), particularly during catch-up growth or weight recovery on energy-dense foods rich in fats and refined carbohydrates, could be implicated in the development of excess adiposity, insulin resistance, compensatory hyperinsulinaemia and an overactive sympathetic nervous system. However, the phenomenon of catch-up fat during catch-up growth/weight recovery still occurs in the absence of hyperphagia (Dullo et al. 2002a), which hence underscores an elevated efficiency for catch-up fat as a fundamental physiological reaction to growth retardation or weight loss. There are therefore close associations between weight recovery/catch-up growth, a high metabolic efficiency underlying catch-up fat, and the development of obesity and/or insulin-related
complications later in life. Consequently, the possibility arises that sustained reduction in energy expenditure per se (because of suppressed thermogenesis in certain organs/tissues) – for the purpose of enhancing the efficiency of fat recovery – is also involved in the pathogenesis of these chronic metabolic diseases.

In this paper, we first review the evidence that an adipose-specific suppression of thermogenesis plays a central role in the phenomenon of catch-up fat. We subsequently concentrate upon recent findings suggesting that: (i) such suppression of thermogenesis directed at catch-up fat is accompanied by a redistribution of glucose from skeletal muscle to adipose tissue, and that (ii) substrate cycling between de novo lipogenesis and lipid oxidation can operate as a thermogenic effector in skeletal muscle in response to signalling interactions between leptin and insulin – two key hormones implicated in the peripheral control of substrate metabolism. These new findings are integrated into the proposal that, in its ‘evolutionary adaptive’ role to spare glucose for the body’s tissues being depleted. Such suppression of thermogenesis, which is observed at a relatively early stage during the course of starvation, is known to be primarily under the control of the sympathetic nervous system (SNS), and is believed to be dictated by signals arising as a direct function of the deficit in food energy (Landsberg et al. 1984, Webber & Macdonald 2000). However, as pointed out above, the common observations that after substantial weight losses or growth retardation, body fat is recovered at a disproportionately faster rate than that of lean tissue (Table 1) also suggests that: (i) a component of the adaptive reduction in thermogenesis during weight loss is also dictated by signals arising specifically from depletion of the fat stores and (ii) it is this component in the adaptive reduction in thermogenesis which persists during weight recovery for the purpose of accelerating the replenishment of fat stores. From a system physiology standpoint, the nature of the adaptive reduction in thermogenesis in response to starvation and refeeding can thus be conceived to be constituted by two distinct control systems (Fig. 1). One control system, which is a direct function of changes in the food energy supply, responds relatively rapidly to the energy deficit. Its effector mechanisms are suppressed early during the

**Adipose-specific control of thermogenesis: from a perspective of system physiology**

It is well documented from longitudinal studies of starvation in humans and rodents that the reduction in basal metabolic rate (BMR) during the progress of starvation is greater than can be accounted for by the loss in body weight and in lean tissues (Keys et al. 1950, Weyer et al. 2000). This deviation from predicted values in the reduction in BMR is generally regarded as the outcome of a regulatory process that, in the face of the starvation stress, increases metabolic efficiency by downregulating cellular energy utilization (i.e. suppresses thermogenesis), and hence reduces the rate at which the body’s tissues are being depleted. Such suppression of thermogenesis, which is observed at a relatively early stage during the course of starvation, is known to be primarily under the control of the sympathetic nervous system (SNS), and is believed to be dictated by signals arising as a direct function of the deficit in food energy (Landsberg et al. 1984, Webber & Macdonald 2000). However, as pointed out above, the common observations that after substantial weight losses or growth retardation, body fat is recovered at a disproportionately faster rate than that of lean tissue (Table 1) also suggests that: (i) a component of the adaptive reduction in thermogenesis during weight loss is also dictated by signals arising specifically from depletion of the fat stores and (ii) it is this component in the adaptive reduction in thermogenesis which persists during weight recovery for the purpose of accelerating the replenishment of fat stores. From a system physiology standpoint, the nature of the adaptive reduction in thermogenesis in response to starvation and refeeding can thus be conceived to be constituted by two distinct control systems (Fig. 1). One control system, which is a direct function of changes in the food energy supply, responds relatively rapidly to the energy deficit. Its effector mechanisms are suppressed early during the

### Table 1 A non-exhaustive list of studies in children and adults reporting a disproportionately faster rate of fat tissue recovery (catch-up fat) with lean tissue recovery lagging behind (adapted from Dulloo et al. 2002a)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Humans showing catch-up fat during weight recovery/catch-up growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kornfeld &amp; Schüller (1931)</td>
<td>Emaciated children recovering from tuberculosis</td>
</tr>
<tr>
<td>Debray et al. (1946)</td>
<td>Prisoners from concentration camps</td>
</tr>
<tr>
<td>Keys et al. (1950)</td>
<td>Men after experimental semi-starvation (Minnesota Experiment)</td>
</tr>
<tr>
<td>Weyer et al. (2000)</td>
<td>Men and women after 2 years limited food supply (Biosphere 2 Experiment)</td>
</tr>
<tr>
<td>Forbes et al. (1984), Mitchell &amp; Truswell (1987)</td>
<td>Anorectics regaining weight</td>
</tr>
<tr>
<td>Barac-Nieto et al. (1979)</td>
<td>Adults after substantial weight loss resulting from protein-energy malnutrition</td>
</tr>
<tr>
<td>Ashworth (1969), MacLean &amp; Graham (1980), Castilla-Serna et al. (1996)</td>
<td>Infants/children showing protein-energy malnutrition</td>
</tr>
<tr>
<td>Van Eys (1985)</td>
<td>Cancer patients</td>
</tr>
<tr>
<td>Streat et al. (1987)</td>
<td>Septic intensive care patients</td>
</tr>
<tr>
<td>Kotler et al. (1990)</td>
<td>AIDS patients – parenteral nutrition</td>
</tr>
<tr>
<td>Jornayvaz et al. (2004)</td>
<td>Children born small-for-gestational age (due to intrauterine growth restriction)</td>
</tr>
</tbody>
</table>
Thermogenesis and insulin resistance

Support for the existence of an adipose-specific control of thermogenesis in humans comes from the reanalysis of data on changes in BMR and in body composition from the Minnesota Experiment (Keys et al. 1950). In this classic study, 32 healthy men of normal body weight were subjected to 24 weeks of semi-starvation during which each man lost approximately 25% of his initial body weight, followed by 12 weeks of restricted refeeding on diets relatively low in fat (~20% fat by energy). Such a link between this slow (adipose-specific) component of the adaptive reduction in thermogenesis and the body’s fat stores was subsequently tested by examining the extent to which inter-individual variability in the change in adjusted BMR (suppressed thermogenesis) could be explained by the degree of depletion of the body’s fat stores. As shown in Figure 2, a plot of $A$-adjusted BMR against the deviation in body fat revealed a positive relation during starvation, i.e. the greater the degree of fat depletion during starvation, the greater the degree of suppression of thermogenesis (Dulloo & Jacquet 1998). A similar relation was also found after the 12-week period of restricted refeeding, i.e. the lower the degree of fat repletion, the greater the extent of reduction in residual BMR and hence the greater the degree of reduction in thermogenesis. These significant correlations between reduced thermogenesis and deviation in body fat contrasted with the lack of correlation against the deviation in fat-free mass, whether during starvation or refeeding (Dulloo & Jacquet 1998). A similar relation between the size of suppression of thermogenesis and the recovery of body fat (and not fat-free mass) has also been reported in patients regaining predominantly fat during rehabilitation from malnutrition caused by non-neoplastic gastrointestinal disease (Carbonnel et al. 1997). Taken together, this continuum in the existence of the relation between suppressed thermogenesis and fat depletion during both phases of weight loss and weight recovery, reflect the operation of a control system with a negative feedback loop between adaptive thermogenesis and the state of depletion of the fat stores, i.e. an autoregulatory feedback system in which signals from the depleted adipose fat stores exert a suppressive effect on thermogenesis.

Evidence from animal studies

More direct evidence for the existence of an adipose-specific suppression of thermogenesis whose role is to accelerate specifically body fat recovery can be derived from complete energy balance studies in laboratory animals regaining weight after semi-starvation (Fig. 3). Under conditions whereby the rehabilitated rats were pair-fed to weight-matched controls, the rate of protein deposition was found to be the same as in controls, but that of fat deposition was increased by more than twofold as a result of 10–15% lower energy expenditure...
during the first 2–3 weeks of isocaloric refeeding (Dulloo & Girardier 1990, Dulloo & Jacquet 2001). A number of factors that could theoretically contribute to this difference in energetics between refed and controls (age difference, physical activity, feeding pattern) have been evaluated and shown to have a minimal impact on the difference in energy expenditure between the two groups (Dulloo & Girardier 1990, 1993). Consequently, under conditions of our refeeding study, the lower energy expenditure in the refed than in the controls is essentially the energy spared as a result of sustained suppression of thermogenesis for the purpose of catch-up fat. The subsequent demonstrations (Dulloo et al. 1995) that when both refed and controls were pair-fed during exposure to cold (a state of markedly elevated sympathetic activation of thermogenesis) the refed animals still showed the capacity for energy conservation directed at catch-up fat, suggested that the mechanisms underlying the adipose-specific suppression of thermogenesis are clearly distinct from sympathetic control of thermogenesis. Viewed in another way, the fact that during weight recovery, suppressed adipose-specific thermogenesis (energy conservation) can co-exist with enhanced SNS-mediated non-specific thermogenesis (energy dissipation), whether in response to stimuli of cold (Dulloo et al. 1995), hyperphagia (Dulloo & Girardier 1993), protein-deficient diets (Dulloo & Girardier 1992) or infections (Arsenijevic et al. 1997), suggest that these two control systems have distinct effector sites, with the adipose-specific control of thermogenesis occurring at sites other than those recruited by the SNS in response to diet and cold.

**Skeletal muscle as a candidate effector site**

Based upon our overall analysis of tracer kinetic studies of noradrenaline turnover rates in various organs and tissues in response to diet or cold (Dulloo & Jacquet 2001), shown in Table 2, we have postulated that the mechanisms underlying non-specific (SNS) control of thermogenesis operate primarily in the metabolically fast-tissues/organs (such as the liver, kidneys, heart and brown fat), and are rapidly restored upon food re-availability. By contrast, the mechanisms underlying the adipose-specific control of thermogenesis – and independently of the SNS – operate primarily in skeletal muscle, a tissue already known to be an important site of starvation-induced suppression of thermogenesis, as judged by studies of regional blood flow by microspheres coupled with measurements of arterial-venous oxygen consumption (Ma & Foster 1986). In other words, the control system underlying the adipose-specific control of
Thermogenesis could operate as a feedback loop between the adipose tissue fat stores and skeletal muscle. As shown in Figure 4, it could hence comprise a sensor(s) of the state of depletion of the fat stores, signal(s) dictating the suppression of thermogenesis as a function of the state of depletion of the fat stores and an effector system mediating thermogenesis in skeletal muscle. In the present state of knowledge, our understanding of the sensor(s), signal(s), and molecular effectors of thermogenesis in skeletal muscle is sketchy. Whatever the molecular physiological mechanisms involved, however, such inter-organ signalling between the size of the fat stores and muscle thermogenesis implies that as skeletal muscle is an important contributor to whole-body energy expenditure and the major site for insulin-mediated glucose disposal, any reduction in this tissue’s metabolic rate would result in a reduction in glucose utilization, thereby leading to hyperinsulinaemia. This in turn, would serve to redirect the spared glucose towards de novo lipogenesis in adipose tissue, a function that coordinates glucose spared for catch-up fat with blood glucose homeostasis.

Table 2  Heterogeneity in sympathetic nervous system (SNS) activity in response to diet and environmental temperature in the rat

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Heart</th>
<th>Pancreas</th>
<th>Liver</th>
<th>Kidney</th>
<th>BAT</th>
<th>WAT</th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold (6°C vs. 22°C)</td>
<td>††</td>
<td>††</td>
<td>○</td>
<td>○</td>
<td>††</td>
<td>††</td>
<td>○†</td>
</tr>
<tr>
<td>Food (fed vs. fasted)</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>○†</td>
</tr>
</tbody>
</table>

BAT, brown adipose tissue; WAT, white adipose tissue.

The thick arrows up or down imply increased or decreased SNS activity as assessed by techniques of radiolabelled noradrenaline turnover in the heart, pancreas, liver, kidney, BAT, WAT and skeletal muscle (for specific references, see Dulloo et al. 2002a); the circle symbols indicate no significant change and thin arrow marginal changes, as assessed by the same technique. Note that the skeletal muscle is not recruited by the SNS in response to diet (and poorly recruited in response to cold), independently of the muscle type studied.
Suppressed thermogenesis and skeletal muscle insulin resistance

Support for this ‘glucose redistribution hypothesis’ can be derived from our recent studies – using the rat model of catch-up fat because of suppressed thermogenesis per se (shown in Fig. 3) – which indicate the following:

1. In response to a glucose load administered intra-peritoneally, plasma insulin concentrations were clearly higher in the refed animals than in controls (Crescenzo et al. 2003);

2. Under hyperinsulinaemic-euglycaemic clamps in vivo, insulin-stimulated glucose utilization in refed animals is lower in skeletal muscle (by 20–43%) but higher in white adipose tissue (by two- to threefold) (Fig. 5); thereby suggesting a state of insulin resistance in skeletal muscle and insulin hyperresponsiveness in white adipose tissue (Cettour-Rose et al. 2005) and;

3. Fatty acid synthase activity is higher in white adipose tissue from refed animals than from fed controls, thereby indicating enhanced conversion

Figure 4 Schematic representation of the adipose-specific control of thermogenesis whose suppression in the skeletal muscle, dictated solely by the state of depletion and repletion of the adipose tissue fat stores, could underlie the elevated efficiency (and hence energy conservation) directed specifically at accelerating fat recovery or catch-up fat. This control system would hence comprise a sensor(s) of the state of depletion of the fat stores, a signal(s) dictating the suppression of thermogenesis as a function of the state of depletion of the fat stores, and an effector system mediating adaptive thermogenesis in skeletal muscle.

Figure 5 Redistribution of glucose from skeletal muscle to adipose tissue during suppressed thermogenesis favouring catch-up fat. Data are for glucose utilization rate (ng min⁻¹ mg⁻¹ tissue), as assessed by hyperinsulinaemic-euglycaemic clamps associated with labelled 2-deoxyglucose, in individual tissues from refed and control rats. All values are mean ± SEM (n = 7–9); levels of statistical significance of differences relative to controls are indicated as follows: *** P < 0.001; ** P < 0.05; * P = 0.08. Adapted from Cettour-Rose et al. (2005).
of glucose to lipids in the fat stores (Cettour-Rose et al. 2005).

Of particular importance in these above-mentioned studies is that this redistribution of insulin-stimulated glucose utilization away from skeletal muscle towards **de novo** lipogenesis in adipose tissue is demonstrated in an experimental setting whereby differences in glucose utilization in muscle and adipose tissue between refed and controls cannot be explained by differences in substrate supply (as both groups consumed the same amount of chow on a day-to-day basis), or in total body fat or circulating free fatty acids (FFAs) as they were not different between refed and control groups at the time-point at which the glucose tolerance test or clamp studies were conducted, i.e. after 7 days of refeeding. Similarly, the state of insulin resistance in skeletal muscle cannot be attributed to excess lipid storage in muscle cells as histological staining of muscles, harvested at the same time-point as for the clamp studies, revealed that intramyocellular lipid content in muscles from refed animals was not higher than in controls. Taken together, these data suggest that muscle insulin resistance and adipose tissue hyperresponsiveness in the refed animals are not related to an excess substrate (FFA) supply or to increased total body fat or ectopic fat storage, but can be linked to the state of suppressed thermogenesis per se.

### Suppressed thermogenesis: from adaptive to maladaptive

These data are thus consistent with the hypothesis that skeletal muscle, which is a major site for glucose disposal in the fed state, might also be a major site for energy conservation (and hence glucose sparing) directed at catch-up fat during weight recovery or catch-up growth. The hyperinsulinaemia and concomitant skeletal muscle insulin resistance during weight recovery would serve to achieve both blood glucose homeostasis and the rapid replenishment of the fat stores, by diverting circulatory glucose away from utilization in skeletal muscle towards **de novo** lipogenesis in adipose tissue. Consistent with this hypothesis is the demonstration that selective inactivation of the muscle insulin receptor gene in mice (which leads to selective insulin resistance in skeletal muscle) also leads to hyperinsulinaemia and promotes redistribution of substrates to adipose tissue, thereby contributing to maintain blood glucose homeostasis while promoting increased adiposity (Kim et al. 2000a). Within the context of weight recovery after growth arrest or after weight loss, such coordinated redistribution of glucose from skeletal muscle utilization to lipogenesis in adipose tissue probably had survival value as it enables the rapid replenishment of fat stores without compromising blood glucose homeostasis under conditions of intermittent periods of food availability that prevailed during much of mammalian evolution. Despite its ‘adaptive’ nature within the context of a lifestyle of famine-and-feast, this state of relatively mild-to-moderate skeletal muscle insulin resistance, adipose tissue hyper-responsiveness and hyperinsulinaemia confers to the phase of weight recovery a greater susceptibility towards the deleterious consequences of a modern lifestyle characterized by low physical activity and energy dense diets rich in fat and refined carbohydrates. In fact, as we previously demonstrated (Crescenzo et al. 2003), a shift in the composition of the diet from complex carbohydrates to animal fat and refined carbohydrates led to a more pronounced state of hyperinsulinaemia, hyperglycaemia and excess adiposity in rats showing weight recovery than in isocalorically-fed controls rats growing spontaneously. As the phase of catch-up growth may last for several months to several years in humans (Keys et al. 1950, Eriksson et al. 2001), these results suggest that the drive to suppressed thermogenesis for the purpose of sparing glucose for catch-up fat, via its orchestration through insulin resistance in skeletal muscle and insulin hyper-responsiveness in adipose tissue, is a central event in the link between catch-up fat, hyperinsulinaemia and risks for later metabolic syndrome.

### Mechanisms linking suppressed thermogenesis with insulin resistance in skeletal muscle

Within this scenario of adaptive suppression of thermogenesis turned maladaptive, diminished thermogenesis in skeletal muscle might therefore be an early event in the pathogenesis of insulin resistance and hyperinsulinaemic state of catch-up fat. Because upon transition from starvation to refeeding, the concentrations of key ‘adiposity’ hormones that might be implicated in the link between glucose metabolism and thermogenesis in skeletal muscle (namely insulin and leptin) are rapidly restored to (or above) control levels, our current working hypothesis is that the suppression of thermogenesis and concomitant insulin resistance in skeletal muscle are brought about through the inhibition of mechanisms through which these hormones interact to activate thermogenesis in skeletal muscle. But what could be the mechanisms that interlink glucose metabolism, lipid oxidation and thermogenesis in skeletal muscle?

### Substrate cycling between **de novo** lipogenesis and lipid oxidation

The fact that leptin is known to activate signal transduction directly in insulin-sensitive organ/tissues
(Kim et al. 2000b) and to stimulate glucose uptake/metabolism (Ceddia et al. 2001), lipid oxidation (Muoio et al. 1997, Minokoshi et al. 2002) or thermogenesis (Dulloo et al. 2002b) in skeletal muscle, prompted us to investigate whether the mechanisms by which leptin’s direct action in skeletal muscle to stimulate substrate metabolism and thermogenesis might be inter-related. Using a method involving repeated measurements of O2 consumption in intact mouse skeletal muscle perfused ex vivo, we recently provided evidence that leptin directly stimulate thermogenesis in skeletal muscle by promoting an energy dissipating substrate cycling between de novo lipogenesis and fatty acid oxidation (Dulloo et al. 2004a, Solinas et al. 2004). Specifically, the direct thermogenic effect of leptin on skeletal muscle O2 consumption was found to be completely inhibited by interference with any one of key control points in the flux of substrates between de novo lipogenesis and mitochondrial β-oxidation (Solinas et al. 2004) as shown in Figure 6, namely by inhibiting either (i) the metabolism of glucose, using 2-deoxyglucose, (ii) the conversion of citrate to acetyl-CoA, using the citrate lyase inhibitor hydroxycitrate, (iii) the conversion of malonyl-CoA to fatty acids, using cerulenin, an inhibitor of fatty-acid synthase, or (iv) the entry of fatty acids into mitochondrial β-oxidation pathway using etomoxir, an inhibitor of CPT-1. It is proposed that during leptin-induced activation of this substrate cycle that links glucose and lipid metabolism to thermogenesis in skeletal muscle, acetyl-CoA produced from fatty acid and glucose oxidation, will overload the Krebs cycle. This will result in excess mitochondrial citrate which, in the cytoplasm, will exert an allosteric activation of the enzyme acetyl-CoA carboxylase (ACC) and at the same time, under the action of citrate lyase, will provide acetyl-CoA to ACC for the synthesis of malonyl-CoA. The latter will serve as the main substrate for fatty acid synthase, thereby producing a new pool of fatty acids. Glucose plays a central role in this cycle as a source of acetyl CoA, Krebs cycle intermediates and NADPH molecules which are

Figure 6 Model illustrating an energy-dissipating ‘futile’ substrate cycling between de novo lipogenesis and lipid oxidation, orchestrated by PI3K (phosphatidylinositol 3-kinase) and AMPK (AMP-activated protein kinase) signalling, in skeletal muscle (see text for details). TG: triglycerides; CPT-1: carnitine palmitoyl transferase-1; ACC: acetyl-CoA carboxylase. The proposed model of substrate cycling is based upon calorimetric studies showing that the direct effect of leptin or corticotropin-releasing hormone (CRH) on skeletal muscle thermogenesis is inhibited by pharmacological interference at any one of key control points in the flux of substrates denoted by symbol (X), namely: (i) with glucose metabolism using 2-deoxyglucose (2-DG), (ii) with the conversion of citrate to acetyl-CoA using hydroxy-citrate, an inhibitor of citrate lyase, (iii) with the conversion of malonyl-CoA to fatty acids using cerulenin, an inhibitor of fatty acid synthase, and (iv) with the entry of fatty acid into mitochondrial β-oxidation pathway using etomoxir, an inhibitor of CPT-1. Adapted from Dulloo et al. (2004a).
required for de novo synthesis of fatty acids (Owen et al. 2002). The capacity of skeletal muscle to perform de novo lipogenesis is supported by the recent demonstrations (Aas et al. 2004, Guillet-Deniu et al. 2004) in muscle satellite cells that glucose stimulates the gene expression of SREBP-1c as well as key genes encoding glycolytic and lipogenic enzymes, leading to an increased lipogenic flux and intracellular lipid accumulation. These findings in primary muscle cell cultures provide direct evidence that de novo lipogenesis can occur in rat and human skeletal muscle cells, and are consistent with data of metabolic labelling from our laboratory (Dulloo et al. 2004a, Solinas et al. 2004), demonstrating that de novo lipogenesis can also occur in intact mouse skeletal muscle, independent of muscle fibre composition.

Requirements for PI3K and AMPK signalling

Subsequent calorimetric studies on these muscle preparations (Solinas et al. 2004) also suggested that this leptin-induced substrate cycling between de novo fatty acid synthesis and fatty acid oxidation could be co-ordinated by signalling pathways involving phosphatidylinositol 3-kinase (PI3K) and AMP-activated protein kinase (AMPK) as:

(1) the thermogenic effect of leptin in the soleus muscle, which is associated with AMPK phosphorylation, can be prevented by inhibitors of AMPK activation using araA, and

(2) the effects of leptin on muscle thermogenesis can also be completely inhibited by inhibitors of PI3K (wortmannin or LY294002), and potentiated by insulin, a potent activator of PI3K.

Thus, in response to leptin, AMPK-induced phosphorylation of ACC, will counterbalance the stimulatory action of citrate on ACC thereby resulting in reduced malonyl-CoA concentration, disinhibition of CPT-1 and increased fatty acid oxidation (Fig. 6). This in turn will lead to the production of acetyl-CoA that will fuel the Krebs cycle, and increase citrate levels. Conversely, PI3K activity, particularly under the influence of insulin, will promote cellular glucose uptake and synthesis of fatty acids. This would occur despite AMPK-induced reduction in malonyl-CoA as it is known that full phosphorylation of ACC by AMPK results in an inhibition of ACC activities only by 50–60% (Winder & Hardie 1996, Gubler et al. 2003). Such partial inhibition of ACC is expected to redirect the flux of acetyl-CoA and malonyl-CoA towards fatty acid oxidation, but would still allow substantial rate of fatty acid synthesis, particularly in presence of high levels of citrate. According to the stoichiometry of this substrate cycle, the synthesis of one molecule of palmitic acid from acetyl-CoA and its re-oxidation to acetyl-CoA would cost at least 14 molecules of ATP. Repeated recycling of acetyl-CoA through the flux of substrates across lipogenesis followed by β-oxidation could therefore constitute an important thermogenic pathway that not only provides a sink for intramuscular glucose disposal, but also for dissipating excess lipids.

Skeletal muscle insulin resistance: via inhibition of substrate cycling?

Besides leptin, one can also entertain the possibility that in skeletal muscle, this substrate cycling is also activated in response to other hormones (e.g. adiponectin, catecholamines) particularly in the light of evidence that adiponectin and adrenergic agonists can also stimulate AMPK activity, glucose utilization and fatty acid oxidation in skeletal muscle (Arch 2002, Minokoshi et al. 2002, Yamauchi et al. 2002). Indeed, our most recent calorimetric studies in skeletal muscle ex vivo suggest that corticotropin-releasing hormone (CRH) – which has been shown to stimulate resting metabolic rate and fat oxidation when infused peripherally in humans (Smith et al. 2001) – directly stimulates thermogenesis in mouse skeletal muscle via substrate cycling between de novo lipogenesis and lipid oxidation, and that the stimulation of this substrate cycle by CRH also requires both AMPK and PI3K signalling (Solinas et al. 2005). Within the context of the link between the adipose-specific suppression of thermogenesis and skeletal muscle insulin resistance during catch-up fat, the possibility therefore arises inhibition of PI3K or AMPK – by hitherto unknown adipose-specific inhibitory ‘lipostatic’ signal – will break down the operation of this thermogenic substrate cycle between de novo lipogenesis and lipid oxidation, with consequential reductions in both fatty acid oxidation and glucose uptake/utilization, thereby providing a mechanism by which suppressed thermogenesis per se predispose to insulin resistance/hyperinsulinaemic state of catch-up fat.

Concluding remarks and perspectives

The notion of interdependency between glucose uptake/metabolism, lipid oxidation and thermogenesis is well recognized at the whole-body level, and is attributed to activation of a neuroendocrine network (comprising insulin, leptin, CRH and the sympathoadrenal system) which play a pivotal role in several overlapping regulatory systems: that of blood glucose, body temperature, body weight and intramuscular lipids. Interference with any of these hormones or their actions lead to major impairments in all these overlapping regulatory functions. In particular, the absence of functional leptin (e.g. in the ob/ob mouse) or dysfunctional leptin signalling
(e.g., in db/db mice or fa/fa rats) leads not only to impairments in thermogenesis that contribute to obesity, but also to hyperglycaemia and excessive accumulation of lipids in non-adipose tissues including in skeletal muscle. The fact that in these animal models, the stimulation of thermogenesis induced by leptin replacement (Unger & Orci 2001) or by treatment with β3-adrenoceptor agonists (Arch 2002) improves insulin sensitivity and reduces intramuscular fat storage at low dose levels or over time-periods that do not affect body weight, strongly suggests that impaired thermogenesis might be an early event in the dysregulation of blood glucose and skeletal muscle substrate metabolism. To date, however, the molecular mechanisms underlying thermogenesis in tissues other than in brown adipose tissue (via the uncoupling protein, UCP1) remains elusive, amid continuing controversies concerning the physiological role of UCP2 and UCP3 (homologues of UCP1) as effectors of skeletal muscle thermogenesis (Samec et al. 1998, Dulloo et al. 2004b). Conversely, increased rate of ATP-consuming ‘futile’ substrate cycles in response to a variety of hormones – including a role for the triacylglycerol/fatty acid cycle in response to leptin (Reidy & Weber 2002) – have been proposed as effectors of thermogenesis (Newsholme 1980), but evidence that they contribute to thermogenesis specifically in skeletal muscle in response to key thermogenic hormone(s) is lacking. The substrate cycle that links glucose and lipid metabolism to thermogenesis in skeletal muscle (Fig. 6) provides a novel molecular mechanism of thermogenesis through which this above-mentioned neuroendocrine network – operating through insulin, leptin, CRH and catecholamines – overlaps in the regulation of body weight, blood glucose and intramyocellular lipids, and hence in the protection against obesity, hyperglycaemia and lipotoxicity. Perturbations in this neuroendocrine network (and intracellular signalling system) that exert control over this substrate cycling may thus be early events that predispose to insulin resistance. It might also provide an effector mechanism by which glucose is spared in skeletal muscle – for redirection towards fat storage – during the phenomenon of catch-up fat. An understanding of how the effector system in skeletal muscle is inhibited as a function of adipose tissue fat stores, how it is modulated by ‘modern’ diets high in fat and refined carbohydrates, and whether it is ‘hypersensitized’ with repeated cycles of weight loss and weight recovery, or by foetal or neonatal programming, are crucial steps towards elucidating the molecular physiological pathways by which suppressed thermogenesis directed at catch-up fat contribute to the pathophysiology of large fluctuations in body weight. In the meantime, it seems important to reiterate that as the phase of catch-up growth (in infants/children) or weight recovery (in adults) may last for months or even years in humans (Keys et al. 1950, Eriksson et al. 2001), the phenomenon of suppressed thermogenesis for the purpose of sparing glucose for catch-up fat, via the coordinated induction of skeletal muscle insulin resistance and adipose tissue insulin hyperresponsiveness, might be a central event in the link between weight fluctuations, hyperinsulinaemia and risks for debilitating diseases that cluster around the insulin-resistance syndrome.

This work is supported by the Swiss National Science Foundation (Grants nos. 3200B0-10215)

References


Thermogenesis and insulin resistance • A G Dulloo


Acta Physiol Scand 2005, 184, 295–307


