Effects of diet on lipolysis and its regulation

BY RICHARD G. VERNON
Hannah Research Institute, Ayr KA6 5HL

The amount of lipid in a fat cell (adipocyte) is determined by the rate of lipolysis as well as the rate of lipid synthesis. Both processes occur simultaneously and continuously, their relative rates determining if there is net lipid loss or accretion.

REGULATIONS OF LIPOLYSIS

Acute control. Hydrolysis of triacylglycerols is catalysed by hormone-sensitive lipase. The activity of this enzyme is enhanced by phosphorylation by cyclic-AMP-dependent kinase (A-kinase). Hormone-sensitive lipase, and hence lipolysis, is thus acutely stimulated by a variety of hormones (e.g. adrenaline, glucagon, adrenocorticotrophic hormone (ACTH)) and the neurohumoral transmitter, noradrenaline, which increase the adenylate cyclase–A-kinase signal transduction system. Each peptide hormone interacts with its own receptor in the plasma membrane while adrenaline and noradrenaline interact with the β-adrenergic receptor. These hormone–receptor interactions lead to dissociation and activation of the GTP-binding protein, Gs, which in turn leads to activation of adenylate cyclase (also located in the plasma membrane) and the synthesis of cyclic-AMP (Fig. 1). Cyclic-AMP activates A-kinase which in turn phosphorylates and activates hormone-sensitive lipase. Signal transduction through this system is modulated by noradrenaline and adrenaline acting via a second receptor, the α2-adrenergic receptor, and by prostaglandins (E1 and E2) and adenosine, both produced within adipose tissue, and acting via their own receptors. These receptors are coupled to a second GTP-binding protein, Gi. Receptor activation leads to dissociation of Gi which inhibits adenylate cyclase (Fig. 1). Catecholamines can, thus, both stimulate and inhibit lipolysis, the net effect depending on the relative numbers of β- and α2-adrenergic receptors of the fat cells.

Insulin also modulates lipolysis acutely. The mechanisms have not been fully elucidated but include activation of a cyclic-AMP phosphodiesterase, possibly by increased phosphorylation (Degerman et al. 1990), which catalyses the degradation of cyclic-AMP and so reduces A-kinase activity. Insulin is also thought to activate the phosphatases which dephosphorylate and inactivate hormone-sensitive lipase (Stralfors & Honnor, 1989).

Recent research is revealing further forms of control for what was once a relatively simple system. A-kinase, and a specific β-adrenergic receptor kinase phosphorylate and uncouple the β-adrenergic receptor from Gs (Roth et al. 1991). A-kinase can also phosphorylate and activate cyclic-AMP phosphodiesterase, providing a feedback loop (Degerman et al. 1990). Another kinase has been discovered which is stimulated by AMP (Hardie, 1989). This kinase phosphorylates hormone-sensitive lipase on a serine next but one to that phosphorylated by A-kinase; phosphorylation on either serine prevents phosphorylation on the other (Garton et al. 1989). Since increased concentrations of palmitoyl-CoA lead to activation of this AMP-stimulated kinase (Hardie, 1989), this
enzyme may also be part of a feedback loop; an accumulation of unesterified fatty acid in
the cell leads to inhibition of lipolysis (Vernon & Clegg, 1985). Blood flow is also
important with respect to this. In blood, fatty acids are bound to albumin. When rates of
lipolysis are high most of the binding sites on albumin may become occupied
so that
removal of fatty acid from the tissue via blood may become limiting, leading to an
accumulation of fatty acids in the tissue and, hence, a diminution in the rate of lipolysis
(Vernon & Clegg, 1985).

Within the fat cell, the lipid droplet is surrounded by a filamentous structure (Slavin,
1972; Franke et al. 1987). It is now becoming apparent that in addition to activation of
hormone-sensitive lipase, mechanisms exist which promote its sequestration into the
membrane-like structure surrounding the lipid droplet and subsequently allow access of
the enzyme to its lipid substrate. For example, disruption of cellular organization by
homogenization can lead to a massive rise in the rate of lipolysis in unstimulated tissue
(Oschry & Shapiro, 1980; Ninomiya et al. 1990) while a protein, termed perilipin, has
been identified on the surface of the fat droplet, which is phosphorylated by A-kinase
(Greenberg et al. 1991) and is postulated to be involved in anchoring hormone-sensitive
lipase to the droplet.

Chronic control. It is now appreciated that the ability of these systems, involved in the
acute control of lipolysis, to transmit signals varies and is subject to chronic endocrine
control. Thyroid hormones, glucocorticoids, sex steroids, growth hormone and insulin
can all exert chronic modulatory effects.

Chronic exposure to growth hormone in vivo (Sechen et al. 1990) and in vitro (Watt

Fig. 1. Lipolytic signal transduction cascade. + , activation; − , inhibition.

\[ \text{Glucagon \rightarrow Glucagon receptor} \]
\[ \text{Catecholamines \rightarrow \beta-receptor} \]
\[ \text{Adenosine \rightarrow Adenosine receptor} \]
\[ \text{Insulin \rightarrow Insulin receptor} \]
\[ \text{Hormone sensitive lipase} \]
\[ \text{Triacylglycerol} \]
\[ \text{Fatty acids, glycerol} \]
et al. 1991) increases response to catecholamines; the mechanism is still unclear, but the hormone can increase β-receptor number (Watt et al. 1989, 1991), cyclic-AMP phosphodiesterase activity and at least one further component downstream of A-kinase (R. G. Vernon and E. Finley, unpublished results). Glucocorticoids also increase β-adrenergic receptor number, but in contrast to growth hormone, decrease maximum adenylate cyclase activity (Giudicelli et al. 1989; Ros et al. 1989). Adrenalectomy increases response to adenosine (Saggerson, 1980; De Mazancourt et al. 1989). Castration in rats and hamsters decreased response to both β- and α2-agonists but had no effect on response to adenosine (Giudicelli et al. 1989; Xu et al. 1991); the decreased response to β-agonists appears to be at least partly due to a reduction in the number of β-adrenergic receptors, and can be reversed by treatment with testosterone (Xu et al. 1991). Ovariectomy reduces response to catecholamines and this can be prevented by oestradiol treatment; the mechanism, however, appears to involve a fall in adenylate cyclase activity with no change in β-receptor number being apparent (Giudicelli et al. 1989; Lacasa et al. 1991).

Hypothyroidism decreases response to β-agonists and increases response to both adenosine and prostaglandin E2 (Malbon et al. 1988). Receptor numbers are unchanged; the effects of adenosine and prostaglandin E2 are at least partly due to increased G1 activity (Milligan & Saggerson, 1990). Hypothyroidism (Engfeldt et al. 1982) and thyroidectomy (Correze & Thibout, 1985) also increase cyclic-AMP phosphodiesterase activity of fat cells. In addition to its acute effects on lipolysis, insulin also appears to have some chronic effects; diabetes, for example, decreases sensitivity to adenosine (Saggerson, 1989) possibly due to a change in G1 activity and decreases adenylate cyclase activity (Strassheim et al. 1990). Diabetes may also alter response to β-agonists and β-receptor number but reports are conflicting (see Vernon, 1992).

**EFFECTS OF DIET**

Diet, either amount or composition, can potentially influence lipolysis in several ways: by altering the serum concentration of acutely acting hormones, by altering sympathetic nervous activity and by altering membrane composition and fluidity or concentrations of chronically acting hormones, or both, thus altering the ability of fat cells to transmit signals. Lipolysis is most sensitive to nutrient availability but dietary composition can also have some effect.

*Nutrient availability: fasting and refeeding.* Fasting or energy restriction alter the serum concentration of a variety of hormones (Table 1) which have implications for lipolysis. However, their relative importance in this respect has been a matter of some controversy which is still not fully resolved. It is well established that serum insulin falls during the postprandial period and remains low during fasting and it is generally agreed that this facilitates lipolysis. Serum glucagon rises on fasting (Unger, 1972; Unger & Orci, 1976; Shetty, 1990) and, hence, should promote the lipolysis of fasting. However, it has been pointed out that glucagon is only weakly lipolytic in a number of species, including man (Hales et al. 1978) and domestic ruminants (Vernon, 1980), and its lipolytic effects are readily suppressed by insulin, hence the quantitative importance of the increase in glucagon for fasting lipolysis remains uncertain.

Catecholamines are strongly lipolytic in mammals and it was thought for a long time
Table 1. Comparison of the effects of fasting and re-feeding, and lactation and litter-removal on some factors which modulate the rate of lipolysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fasting</th>
<th>Refeeding</th>
<th>Lactation</th>
<th>Litter-removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum insulin</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Serum glucagon</td>
<td>↑</td>
<td>?</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Serum adrenaline</td>
<td>↑</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Serum triiodothyronine</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>Sympathetic nervous activity</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>?</td>
</tr>
<tr>
<td>Adipocyte</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to adrenaline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↓</td>
</tr>
<tr>
<td>Sensitivity to adrenaline</td>
<td>↑</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-adrenergic receptor number</td>
<td>↑</td>
<td>?</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Adenylate cyclase activity</td>
<td>-</td>
<td>↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal</td>
<td>-</td>
<td>↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maximum</td>
<td>-</td>
<td>?</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>Cyclic-AMP phosphodiesterase activity</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>-</td>
</tr>
</tbody>
</table>

↓, ↑, -., Concentration/activity decreased, increased or the same respectively as that found at onset of postprandial period.

that fasting must enhance sympathetic nervous activity, thereby stimulating lipolysis. However, it now appears that sympathetic nervous activity in general is decreased during fasting, although this has not been shown specifically for white adipose tissue (Barrand & Callingham, 1983; Landsberg & Young, 1985); a decrease in sympathetic activity is perhaps not surprising as it results in a lower metabolic rate thus conserving reserves of nutrients. However, while increased noradrenaline release from nerve endings can probably be excluded as a major lipolytic promoter during fasting, it is now apparent that adrenaline release from the adren medulla is under separate control (Young et al. 1984). A number of studies (e.g. Young et al. 1984; Wolfe et al. 1987; Klein et al. 1989), but not all (Jung et al. 1979), report increased plasma adrenaline concentrations during fasting and a recent study shows that this increase begins during the postprandial period (Coppack et al. 1990). In addition several studies have reported that β-adrenergic blockade (Kozlowski et al. 1985; Klein et al. 1989) or surgical denervation (Bray & Nishizawa, 1978) during fasting prevents the rise in serum free fatty acids and glycerol (used as indices of increased lipolysis). Some earlier studies argued against a role of adrenaline in fasting-induced lipolysis (see Shetty, 1990) but the weight of recent evidence would suggest that adrenaline is of importance. Thus, it is probable that a combination of increased serum adrenaline, and perhaps glucagon, and decreased insulin concentrations all contribute to the enhanced lipolysis during a fast. The effect of fasting on local modulators of lipolysis (adenosine and prostaglandin E2) in adipose tissue extracellular fluid is unknown, but developments in microdialysis (e.g. Lonnroth et al. 1989) should soon provide an answer to this.

Several studies have shown that fasting increases the response and sensitivity of lipolysis to catecholamines in man in vivo (Arner et al. 1981a; Jensen et al. 1987; Wolfe et al. 1987; Klein et al. 1990); this is a chronic adaptation developing between about 15 h
and 4 d of fasting. The factors responsible have not been elucidated, and while a fall in serum insulin and glucose contribute to the enhanced lipolytic response to catecholamines, they do not provide a complete explanation (Jensen et al. 1987; Klein et al. 1990). Also, paradoxically, an increased response to the anti-lipolytic effect of adenosine has been proposed (Peters et al. 1991).

In vitro studies with isolated fat cells or tissue pieces also reveal an increased sensitivity to catecholamines on fasting in man (Andrews, 1984), hamsters (Carpene et al. 1990) and rats (Zapf et al. 1977; Dax, 1981; Chohan et al. 1984). In addition Saggerson (1986) reported an increased sensitivity to glucagon but not ACTH in fat cells from fasted rats. Fasting did not increase maximum response to catecholamines in isolated fat cells from humans (Andrews et al. 1984), in perfused canine adipose tissue (Fredholm et al. 1973) and in some studies with rat (Zapf et al. 1977; Dax et al. 1981; Saggerson, 1986) fat cells. Another study with rats suggests that fasting results in a reduced response to noradrenaline (Giudicelli et al. 1982). This apparent discrepancy is probably due to a change which develops as fasting proceeds, with a decrease in response occurring around 72 h of fasting. This is supported by a detailed study with hamsters in which fasting had no effect on maximum response during the first 48 h of fasting but by 72 h of fasting a decreased response was apparent (Carpene et al. 1990). The apparent discrepancy between effects of fasting on maximum response to catecholamines in vivo and in vitro in humans may be due to the ethical problem of achieving a maximum response in man in vivo.

The mechanism responsible for the enhanced sensitivity of fat cells to catecholamines during fasting has not been elucidated. Unfortunately most studies with rats have involved 72 h of fasting at which time the response to catecholamine is beginning to decrease. Fasting for 72 h increased the β-receptor number of rat fat cells (Giudicelli et al. 1982). Fasting for 48 h (Gorman et al. 1972) or 72 h (Dax et al. 1981) increased catecholamine-stimulated adenylate cyclase activity of rat fat cells. Maximum adenylate cyclase activity did not appear to change after 72 h fasting in rats (Giudicelli et al. 1982), but the ability of Gα to transmit signals appeared to be enhanced (Lacasa et al. 1986a). Fasting for 72 h decreased adipose tissue cyclic-AMP phosphodiesterase activity in rats while activation by insulin appeared to be unimpaired (Osegawa et al. 1985). Also cyclic-AMP appears to be less effective in stimulating lipolysis in femoral subcutaneous (but not hypogastric) adipocytes during therapeutic starvation in obese humans (Arner et al. 1981b). Thus, a number of adaptations appear to contribute to the enhanced sensitivity to catecholamines during fasting. In addition to these various biochemical adaptations, fasting also causes profound morphological changes within the fat cell including increased numbers of vesicles within the cytosol (Mizunuma et al. 1981); the role of these vesicles and other morphological adaptations is uncertain but may be involved in facilitating access of hormone-sensitive lipase to its substrate.

The decreased response to catecholamines found on prolonged fasting noted previously may be due to a decrease in fat cell size (Arner et al. 1979) or the development of hypothyroidism, or both. The molecular mechanism responsible for the diminished response to catecholamines has not been elucidated, but does not appear to involve a loss of β-adrenergic receptors or adenylate cyclase activity, at least in rats (Giudicelli et al. 1982) indicating a defect in the distal portion of the signal transduction system.

In addition to changes in the stimulatory system, fasting decreases the responsiveness of the α2-adrenergic system in hamster adipocytes, in part at least, due to a decrease in the number of α2-adrenergic receptors (Carpene et al. 1990). In vitro studies with rats
show a decreased sensitivity to both adenosine and prostaglandin E₁ on fasting for 24 or 48 h (Chohan et al. 1984; Saggerson, 1986), but no change in the number or affinity of adenosine receptors (Chohan et al. 1984). On the other hand, Lacasa et al. (1986b) found no evidence for altered ability of adenosine to inhibit adenylate cyclase activity in adipocytes from rats fasted for 72 h. Also Carpene et al. (1990) found the response to adenosine and prostaglandin E₁ unchanged after a 6 d fast in hamsters. In man, fasting for 4 d may induce an increased response to adenosine (Peters et al. 1991), whereas Kather et al. (1985) found no evidence for a change in response or sensitivity to either adenosine or prostaglandin E₂ in response to energy restriction in obese humans.

Thus, the regulation of lipolysis passes through several phases during fasting. Changes in acute signals (e.g. the fall in serum insulin, and rise in serum glucagon and adrenaline concentrations) occur early on within the first few hours of a fast. The times when the increased sensitivity to catecholamines and glucagon develop are unknown but are clearly chronic, occurring about 24 h or more after the onset of fasting. In rats, but seemingly not in man, there is initially at least a decreased sensitivity to adenosine and prostaglandins. Prolonged fasting, perhaps due to the development of hypothyroidism, results in a diminished lipolytic response to catecholamines, which could be perceived as part of a general mechanism to minimize nutrient utilization and so prolong survival.

Feeding induces a rapid rise in serum insulin and a decrease in serum glucagon (Unger, 1972; Unger & Orci, 1976) and adrenaline but no change in noradrenaline (Coppack et al. 1990). Feeding rapidly suppresses lipolysis as assessed from arterio-venous difference measurements of both glycerol and fatty acid release in man (Coppack et al. 1990). In rodents, feeding results in an increase in sympathetic nervous activity in brown adipose tissue at least (this has not been checked in white adipose tissue; Landsberg & Young, 1985; Barrand & Callingham, 1987) and an increase in triacylglycerol turnover in white adipose tissue in vivo which can be prevented by the administration of β-adrenergic antagonists (Brooks et al. 1983); that is, there is a small surge in lipolysis in rodents but with essentially all the fatty acids being re-esterified rather than released into the circulation (Suzuki et al. 1983).


**Nutrient availability: lactation cycle.** Although lactation is a state of markedly elevated food intake, for much of the body it is a state of effective deprivation and in many respects resembles fasting (Table 1). Fat synthesis in adipose tissue is markedly reduced and there is usually a net loss of adipose tissue lipid (Vernon & Flint, 1984). During lactation serum glucagon does not change, serum growth hormone may rise and serum insulin, glucose and triiodothyronine concentrations are all decreased (Vernon, 1989) and in brown adipose tissue at least there is decreased sympathetic nervous activity (Trayhurn & Wusteman, 1987), all reminiscent of the fasting state. In ruminants lactation may result in an enhanced response to catecholamines and an increase in the number of β-adrenergic receptors (Vernon & Sasaki, 1991) possibly due to increased serum growth hormone levels (Watt et al. 1991), but in the rat response and sensitivity to catecholamines is unchanged (Vernon & Flint, 1984). Cyclic-AMP phosphodiesterase activity falls during lactation in rats (Aitchison et al. 1982) and the number of β-adrenergic receptors increases (Watt et al. 1989), both of which might be expected to
increase the response or sensitivity to catecholamines. Recently we have found that the maximum adenylate cyclase activity falls during lactation and this appears to counteract the effects of the other adaptations, because the ability of catecholamines to activate A-kinase is unchanged.

Removal of the litter from lactating rats has similar effects to re-feeding fasting rats in that serum insulin and glucose concentrations are restored and there is a massive increase in the rate of lipid synthesis in adipose tissue (Vernon & Flint, 1984). However, in contrast to re-feeding fasted rats, litter removal results in a marked decrease in the response (but not sensitivity) of lipolysis to catecholamines which can be prevented by treatment with growth hormone (Vernon et al. 1987). Litter removal results in a fall in the number of β-adrenergic receptors of fat cells (Watt et al. 1989; Ros et al. 1992), restoration of adenylate cyclase activity and an increase in cyclic-AMP phosphodiesterase activity (R. G. Vernon, P. W. Watt and E. Finley, unpublished results). Despite these changes, the ability of catecholamines to activate A-kinase is unaltered, indicating that the main constraint is downstream of A-kinase. Our recent results have shown that this constraint does not result from changes in protein phosphatase activity or AMP-stimulated kinase activity which might influence hormone-sensitive lipase activity. Recent results show that there is no change in measurable hormone-sensitive lipase activity in adipose tissue homogenates made from tissue after exposure to catecholamines, but the distribution of the enzyme in the cell is altered, with less being associated with the fat cake in tissue from litter-removed rats (L. Piperova and R. G. Vernon, unpublished results). This suggests an important control point at the level of hormone-sensitive lipase sequestration on to the lipid droplet or access to its lipid substrate, or both.

Lactation, curiously, also results in an increased response to adenosine which gradually decreases on litter removal (Vernon & Finley, 1986). The physiological advantage of this is uncertain but it may be a device to prevent the animal depleting its lipid reserves too rapidly. The hypothyroidism of lactation is a possible cause but preliminary studies do not support this. Growth hormone, however, accelerates restoration of normal response to adenosine during litter removal (Vernon et al. 1987).

**Dietary composition.** Serum insulin concentration varies directly with the carbohydrate content of the diet, whereas serum glucagon tends to decrease as the carbohydrate content increases (Unger & Orci, 1976). Such variations, however, are small compared with those found on fasting. Sympathetic nervous activity in white adipose tissue, on the other hand, appears to be impervious to the relative amount of fat and carbohydrate in the diet but is increased by low-protein diets (Vander Tuig & Romso, 1984). The nature of dietary carbohydrate does not appear to alter sympathetic nervous activity, at least in brown adipose tissue (Walgren et al. 1987). Whether these responses to dietary composition have any significance for lipolysis is not clear.

Diet can also alter the ability of fat cells to transmit signals. Feeding rats diets containing a large amount of saturated fat (usually lard) results in a smaller maximum lipolytic response to catecholamines but no change in sensitivity compared with animals fed on carbohydrate-rich diets (Gorman et al. 1972, 1973; Smith et al. 1974; Susini et al. 1979; Tepperman et al. 1986) and a decreased response to the anti-lipolytic effect of insulin (Smith et al. 1974) in one study but not in others (Susini et al. 1979; Tepperman et al. 1986). Gorman et al. (1973) found no change in ligand binding to the β-receptor or in maximum basal adenylate cyclase activity but there was a diminished ability to activate
adenylate cyclase by adrenaline in fat cells from fat-fed rats suggesting a limitation at the level of G.

Studies with human adipose tissue revealed no differences in response or sensitivity to catecholamines after eating fat-rich or carbohydrate-rich diets for 7 d (Kather et al. 1987); in this study, in contrast to those with rats, the authors found that the fat-rich diet enhanced the response to the anti-lipolytic effect on insulin.

Other studies suggest that it is the nature of the fatty acids rather than the amount of fat in the diet which is important, as diets rich in polyunsaturated fats result in a much greater lipolytic response to catecholamines than diets rich in saturated fat (Carreau et al. 1972; Awad & Zepp, 1979; Awad & Chattopadhyay, 1986; Parrish et al. 1991). Decreased adenylate cyclase, cyclic-AMP phosphodiesterase and hormone-sensitive lipase (Awad & Chattopadhyay, 1986) may all contribute to the decreased responsiveness of fat cells from rats fed on diets rich in saturated fats. Changes in adenylate cyclase at least may be due to changes in membrane fluidity (Nicolas et al. 1990). Changes in membrane fluidity also alter insulin binding and action in adipocytes (Clandinin et al. 1991) and β-receptor number in other tissues at least (Peters, 1988; Murphy, 1990).

CONCLUSIONS

The rate of lipolysis is, thus, subject to a surprisingly large range of acute and also chronic controls, which may be altered by the nutrient availability (energy status) and to a lesser extent by dietary composition, particularly by diets containing a large amount of saturated fat. Nutrient availability or composition is likely to influence lipolysis initially by altering the concentration of acute modulators, but can also lead to changes in response or sensitivity to these acutely acting factors through changes in chronically acting hormones or membrane fluidity, or both. Adaptations may occur in both the stimulatory pathways and in the inhibitory (e.g. adenosine) pathways, and in general do not appear to involve a change in a single component, rather the activity or amount of several components may be altered, sometimes paradoxically with counteracting effects. The chronically-acting hormones responsible for these adaptations of the signal transduction systems have not been resolved. As well as biochemical changes, changes in energy balance can also have marked effects on adipocyte morphology, the physiological significance of which is not yet understood.

REFERENCES


THE MANIPULATION OF ADIPOSY


