

Sir David Cuthbertson Medal Lecture

Regulation of lipid metabolism in adipose tissue

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Adipose tissue is a major source of metabolic fuel. This metabolic fuel is stored in the form of triacylglycerol. Lipolysis of triacylglycerol yields non-esterified fatty acids and glycerol. In human subjects *in vivo* studies of the regulation of lipid metabolism in adipose tissue have been difficult because of the heterogeneous nature of the tissue and lack of a vascular pedicle. In the last decade the methodology of study of adipose tissue has improved with the advent of the anterior abdominal wall adipose tissue preparation technique and microdialysis. These techniques have demonstrated that lipid metabolism in adipose tissue is finely coordinated during feeding and fasting cycles, in order to provide metabolic fuel when required. Lipolysis takes place both in extracellular and intracellular space. The extracellular lipolysis is regulated by lipoprotein lipase and the intracellular lipolysis is regulated by hormone-sensitive lipase. In pathophysiological conditions such as trauma, sepsis and starvation profound changes are induced in the regulation of lipid metabolism. The increased mobilization of lipid fuel is brought about by the differential actions of various counter-regulatory hormones on adipose tissue blood flow and adipose tissue lipolysis through lipoprotein lipase and hormone-sensitive lipase, resulting in increased availability of non-esterified fatty acids as a source of fuel. In recent years, it has been demonstrated that adipose tissue produces various cytokines and these cytokines can have paracrine and endocrine effects. It would appear that adipose tissue has the ability to regulate lipid metabolism locally as well as at distant sites such as liver, muscle and brain. In future, it is likely that the mechanisms that lead to the secondary effects of lipid metabolism on atheroma, immunity and carcinogenesis will be demonstrated.

Adipose tissue: Lipid metabolism: Counter-regulatory hormones: Cytokines

In the first half of this century adipose tissue received little attention, as it was thought to be an organ of fat storage lacking any specific metabolic activity (Wertheimer & Shapiro, 1948). This view remained prevalent until the work of Gordon & Cherkes (1956) brought attention to the possible role of adipose tissue as a source of metabolic fuel. It was Cahill (Cahill *et al.* 1966), whose seminal work on fuel utilization during prolonged starvation established the role of adipose tissue as the main source of metabolic fuel. In an adult adipose tissue may make up 20–25 % of the whole-body weight, yet this tissue can account for more than 85 % of the total stored energy (Cahill, 1970). Oxidation of fat yields twice as much energy as that of carbohydrate or protein. Protein and carbohydrate

(glycogen) storage requires water. Fat can be stored more efficiently because of its hydrophobic nature, as it does not require water for storage.

Lipid metabolism is inextricably linked to carbohydrate and protein metabolism; therefore, adipose tissue plays a part in carbohydrate and protein metabolism. Adipose tissue takes up glucose and releases lactate. In addition, it releases glycerol, alanine and glutamine, all of which can act as substrates for gluconeogenesis (Frayn *et al.* 1991). In recent years it has been demonstrated that adipose tissue releases a number of cytokines, prominent among which are tumour necrosis factor (Hotamisligil *et al.* 1993), interleukin 6 (Mohamed-Ali *et al.* 1997), leptin (Considine *et al.* 1996) and plasminogen activator inhibitor-1 (Samad *et al.* 1996).

Abbreviations: ATBF, adipose tissue blood flow; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids; TAG, triacylglycerols.

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Adipose tissue can also form active hormones such as oestradiol and cortisol from their precursors (Boulton *et al.* 1992; Katz *et al.* 1999). In addition, it possesses monoamine oxidase that can metabolize catecholamines (Samra *et al.* 1996d). The main function of adipose tissue remains regulation of lipid metabolism.

Methods of study of lipid metabolism in adipose tissue *in vivo*

In order to study the regulation of metabolism in any tissue a relatively pure preparation of that tissue is required. In addition, to measure the exchange of substances its vascular pedicle must be accessible. The distinct lack of a pure adipose tissue preparation in human subjects remained a stumbling block for the study of the regulation of lipid metabolism in adipose tissue. It was in the late 1980s when Frayn *et al.* (1989) described a relatively pure preparation of adipose tissue that could be studied by the arterio-venous technique. They were able to show that the subcutaneous adipose tissue of the anterior abdominal wall was drained by the superficial inferior epigastric vein. There was no contamination from the underlying muscle, as the adipose tissue was separated by fibrous tissue and the contribution of overlying skin was minimal. The main limitation of this technique is that only one depot of adipose tissue can be studied.

In the late 1980s Lönnroth *et al.* (1987) developed the technique of adipose tissue microdialysis. A microdialysis probe is inserted into the tissue of interest. This probe has a semi-permeable component which sits within the tissue of interest and thus allows the exchange of substances between the infusate and interstitial fluid. The interstitial concentration of a given substance can be measured by varying the infusate concentrations of the substance of interest and calculating the dialysate concentration of that substance. If a graph is plotted of the difference between the dialysate and infusate concentration *v.* the infusate concentration, the interstitial concentration must be where the line crosses the *x* axis, i.e. the infusate concentration at which the difference between the infusate and dialysate concentration must be zero. The slope of the graph gives the recovery of the substance for that probe. The second and most-commonly-used method is to reduce the rate of infusate to such a low value that the recovery of substance is complete. It has been shown that this situation can be achieved by infusing at a rate lower than 0.3 $\mu\text{l}/\text{min}$. A unique insight can be gained into the adipose tissue interstitial environment by using this technique (Samra *et al.* 1996c). The venous concentration of a given substrate can be calculated from the interstitial concentration and its permeability constant and plasma water flow (Summers *et al.* 1998). Once the venous concentration is calculated, Fick's principle (Fick, 1872) can be applied to calculate the exchange of a given substance. The main advantage of this technique is that any depot of adipose tissue can be studied. The disadvantage is that hydrophobic and large lipoprotein molecules cannot be studied by this technique.

The use of the microdialysis and adipose tissue venous cannulation technique has significantly improved our understanding of the *in vivo* regulation of lipid metabolism in

human adipose tissue. Adipose tissue blood flow (ATBF) can potentially play an important role in lipid metabolism, as it can alter the rate of delivery or removal of substrates from adipose tissue. In order to study the quantitative exchange of substrates to and from adipose tissue it is essential that ATBF can be calculated accurately. In the past the NaI detector has been used to determine the washout of ^{133}Xe from adipose tissue in order to calculate ATBF. The movement of the adipose tissue relative to the detector can introduce large errors in the calculation of ATBF. Thus, a new lightweight detector with a CsI crystal has been developed to study ATBF. The use of this detector has shown that ATBF can change during feeding (Samra *et al.* 1995) and simulated stress (Samra *et al.* 1996d). The importance of ATBF in the role of lipid metabolism should not be underestimated, as its dysregulation has been postulated to feature in or potentially to be the cause of obesity and insulin resistance state (Summers *et al.* 1999).

In adipose tissue lipids are stored in adipocytes as a large fat droplet, most of which is formed by triacylglycerol (TAG). A TAG molecule is composed of a molecule of glycerol and three fatty acid molecules. In the vascular compartment lipid fuel exists either in the form of non-esterified fatty acids (NEFA) bound to albumin, because of their hydrophobic nature, or as lipoproteins. Lipoproteins are large molecules; their central core consists of hydrophobic TAG molecules and their surface is formed from hydrophilic phospholipid and apolipoprotein molecules. The different value for TAG:protein and different apolipoproteins lead to increased diversity and complexity in lipoprotein metabolism.

Regulation of lipid metabolism during feeding and fasting cycle

After a meal the enterocytes synthesize TAG from NEFA and the TAG are secreted in the form of chylomicrons. A chylomicron is a large lipoprotein which has a core rich in TAG. When a chylomicron molecule passes through the adipose tissue capillary its TAG are degraded by lipoprotein lipase (LPL). This LPL-induced intravascular lipolysis in adipose tissue is regulated by the plasma insulin concentration. After a meal the rising plasma concentration of insulin results in increased activity of adipose tissue LPL. The liberated exogenous NEFA are directed into adipocytes as the rising plasma insulin concentration inhibits the intra-adipocytic lipolysis of TAG by decreasing the activity of hormone-sensitive lipase (HSL; Fig. 1). There has been a considerable debate as to how the LPL-liberated NEFA are captured by adipose tissue. It has been postulated and confirmed by *in vitro* studies that the LPL-liberated NEFA are directed into adipocytes preferentially under the influence of acylation-stimulating protein (Saleh *et al.* 1998). In addition, the increasing plasma insulin concentration leads to increased uptake of glucose by the adipocytes. The ensuing intra-adipocytic glycolysis leads to increased capture of endogenous NEFA by glycerol-3-phosphate and formation of intra-adipocytic TAG. The increased capture of NEFA can be gauged from NEFA:glycerol release, as glycerol cannot be metabolized by adipose tissue. A NEFA:glycerol value of less than 3:1

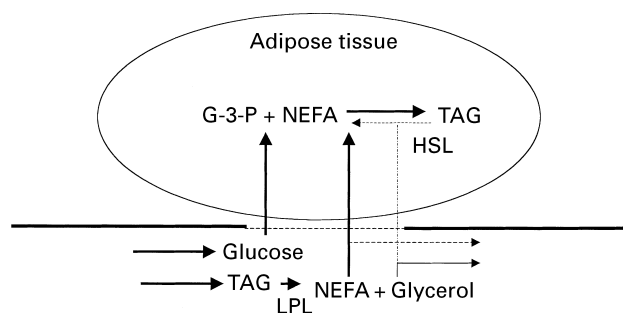


Fig. 1. Regulation of lipid metabolism in adipose tissue in the post-absorptive period. There is net uptake of non-esterified fatty acids (NEFA) by the adipose tissue under the influence of lipoprotein lipase (LPL). In addition, the reduction in hormone-sensitive lipase (HSL) activity and increased primary re-esterification of NEFA leads to retention of NEFA in adipose tissue. G-3-P, glycerol-3-phosphate; TAG, triacylglycerols; \longrightarrow , major activity; $-\ - \longrightarrow$, minor activity.

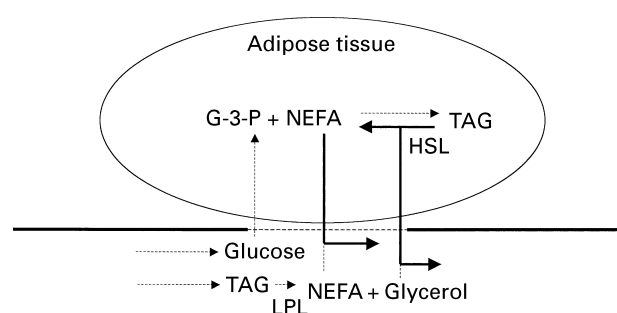


Fig. 2. Regulation of lipid metabolism in adipose tissue in the fasting state. Adipose tissue becomes net exporter of non-esterified fatty acids (NEFA) as the lipoprotein lipase (LPL) activity decreases, the hormone-sensitive lipase (HSL) activity increases. The increased mobilization and the reduction in re-esterification of NEFA within adipose tissue results in net efflux of NEFA from adipose tissue. G-3-P, glycerol-3-phosphate; TAG, triacylglycerols; \longrightarrow , major activity; $-\ - \longrightarrow$, minor activity.

indicates the increased capture or primary re-esterification of NEFA. Adipose tissue LPL activity cannot be measured *in vivo*; however, its rate of action can be determined from TAG uptake by the following equation:

$$\text{LPL rate of action} = (a-v)_{\text{TAG}} \times \text{ATBF},$$

where $(a-v)_{\text{TAG}}$ is the arterio-venous difference for TAG.

In the postprandial to fasting state, the fall in plasma insulin concentrations leads to decreased adipose tissue LPL activity, resulting in diminished intravascular lipolysis of TAG. More importantly, the fall in plasma insulin concentration leads to increased activity of HSL, resulting in increased intra-adipocytic TAG hydrolysis. In addition, the reduced uptake of glucose by the adipocytes leads to a reduction in intra-adipocytic glycolysis, resulting in decreased formation of glycerol-3-phosphate. Adipose tissue becomes a net exporter of NEFA as a result of increased HSL activity and reduced primary re-esterification (Fig. 2).

The activity of HSL can be reflected by the rate of action of HSL, which can be calculated by stoichiometric analysis:

$$\text{HSL rate of action} = [(v-a)_{\text{glycerol}} - (a-v)_{\text{TAG}}] \times \text{ATBF},$$

where $(v-a)_{\text{glycerol}}$ is the veno-arterial difference for glycerol and $(a-v)_{\text{TAG}}$ is the arterio-venous difference for TAG.

It would appear that the net flow of NEFA within adipose tissue is precisely controlled to produce efflux of NEFA during the fasting state and retention during the fed state. This control is brought about by the coordinated regulation of HSL and LPL (Frayn *et al.* 1995). The efflux of NEFA from adipose tissue and the transcapillary efflux of NEFA can also be calculated by using the arterio-venous technique:

$$\text{efflux of NEFA} = (v-a)_{\text{NEFA}} \times \text{ATBF},$$

$$\text{transcapillary efflux of NEFA} = [(v-a)_{\text{NEFA}} - 3(a-v)_{\text{TAG}}] \times \text{ATBF},$$

where $(v-a)_{\text{NEFA}}$ is the veno-arterial difference for NEFA and $(a-v)_{\text{TAG}}$ is the arterio-venous difference for TAG.

The arterial concentration of NEFA is regulated by the subcutaneous adipose tissue veno-arterial differences for NEFA (Frayn *et al.* 1989; Samra *et al.* 1996a). The NEFA released by the adipose tissue are either taken up by the muscle and oxidized, or they are removed by the liver. The whole-body oxidation of NEFA is directly related to their arterial plasma concentration (Groop *et al.* 1991). In addition, the release of NEFA by the adipose tissue also determines the uptake of NEFA by the liver (Bülow *et al.* 1999). In the liver NEFA can be oxidized, converted into ketones, or reformed into TAG and secreted as VLDL. It has been postulated that muscle LPL has greater avidity for VLDL-TAG than adipose LPL. The NEFA liberated by VLDL-TAG can either be removed by muscle for oxidation or recycled back to the liver to be hydrolysed by LPL liberating NEFA. The LPL activity in muscle is reciprocally linked to adipose LPL activity. This interaction increases the efficiency of the lipid utilization and reflects the close coordination of lipid metabolism by liver, adipose tissue and muscle.

Regulation of lipid metabolism during starvation

Starvation is a process during which well-defined changes in the utilization of metabolic fuel occur in order to enhance survival. These changes are well integrated with the feeding and fasting cycle, so as to provide a continuous supply of metabolic fuel. In a normal feeding and fasting cycle glucose and NEFA are the main source of metabolic fuel. However, if the fast continues, carbohydrate stores become depleted within the first 24 h, and NEFA from adipose tissue become the main source of metabolic fuel along with amino acids (Owen *et al.* 1998). Over the next few days of fast amino acid oxidation diminishes as cells such as neurones start using ketones as an alternative fuel provided by NEFA. Stable-isotope turnover studies have demonstrated that maximum change in lipid metabolism occurs between 12 and 24 hours of starvation (Klein *et al.* 1993). Adipose

tissue arterio-venous studies have demonstrated that after an overnight fast one-quarter of the NEFA efflux from adipose tissue can still be accounted for by the action of adipose tissue LPL. During early starvation (14–20 h of fast), the rate of LPL action decreases and yet the net efflux of NEFA from adipose tissue increases. This increased efflux of NEFA from adipose tissue can be accounted for by the increased transcapillary release of NEFA within adipose tissue. The rise in transcapillary efflux of NEFA is due to increased HSL rate of action (Samra *et al.* 1996a). The reduction in the re-esterification of NEFA within adipocytes plays no part in their increased efflux from adipose tissue, as after an overnight fast there is very little re-esterification. After an overnight fast the basal tone of lipolysis is regulated by catecholamines (Arner *et al.* 1990), cortisol (Samra *et al.* 1996b) and growth hormone (Samra *et al.* 1999), but the increasing rate of action of HSL appears to be predominantly due to decreasing levels of plasma insulin concentration. The release of individual plasma NEFA from subcutaneous adipose tissue broadly determines the composition of plasma NEFA. There are differences in the mobilization of each NEFA relative to its adipose tissue content: for a given chain length, the relative mobilization increases with increasing unsaturation, and for a given degree of unsaturation the relative mobilization decreases with increasing chain length (Halliwell *et al.* 1996).

Increased lipolysis in adipose tissue during starvation also provides increased glycerol as a substrate for gluconeogenesis. After an overnight fast 4–10 % of the whole-body glucose production can be accounted for by glycerol, and after 4 d of starvation this contribution increases to 20–25 % (Baba *et al.* 1995).

In starvation the survival of the host depends on the tight and well-co-ordinated regulation of lipid metabolism in adipose tissue. The period for which an individual can tolerate starvation in the absence of sepsis or trauma depends on the size of adipose tissue depots.

Regulation of lipid metabolism during sepsis and trauma

Lipids are the preferred fuel for oxidation in patients with sepsis (Shaw & Wolfe, 1987) and trauma (Stoner *et al.* 1979; Wolfe *et al.* 1987). This rise in lipid oxidation is directly attributable to the increased plasma NEFA concentration. The mechanisms that lead to increased NEFA mobilization during sepsis and trauma are complex and poorly understood (Samra *et al.* 1996e). Trauma and sepsis initiate cytokine and hormonal cascades, and these factors in turn have profound effects on lipid metabolism.

In both sepsis and trauma plasma cytokines such as tumour necrosis factor- α , interleukins 1 β and 6 can increase rapidly (Michie *et al.* 1988; Rouman *et al.* 1993). *In vivo* studies have demonstrated that tumour necrosis factor- α and interleukin 6 infusion can cause profound changes in plasma adrenaline, noradrenaline and cortisol concentrations (van der Poll *et al.* 1991; Stouthard *et al.* 1995). These cytokines can have direct effects on liver, muscle and adipose tissue lipid metabolism, and more importantly they can have indirect effects on lipid metabolism through changes in plasma counter-regulatory hormones. The cytokine

response to trauma and sepsis can be variable, depending on the nature of the assault. The hormonal infusion studies simulating stress have been useful in providing an insight into the mechanisms that regulate substrate metabolism (Gelfand *et al.* 1984). However, it has to be appreciated that while hormonal infusion studies may provide insight into various regulatory mechanisms they have not been able to account for all the metabolic effects seen in sepsis and trauma (Frayn, 1986).

In human subjects adrenaline infusion leads to increased efflux of NEFA from adipose tissue; this increase is reflected by their increased arterial concentration. The increased efflux of NEFA from adipose tissue is accounted for both by the increased transcapillary efflux of NEFA as a result of increased intra-adipocytic lipolysis and by increased adipose tissue intravascular lipolysis (Samra *et al.* 1996d). Adrenaline causes a marked rise in ATBF, which may explain the discrepancy seen in the LPL activity between *in vitro* studies and *in vivo* studies. The higher ATBF results in increased presentation of lipoprotein-TAG as a substrate for LPL, to be hydrolysed in the vascular compartment. In addition, the increased ATBF prevents local accumulation of products of lipolysis. Furthermore, there is an uptake of adrenaline by adipose tissue, thus confirming the possibility of the presence of monoamine oxidase in adipose tissue. The effect of noradrenaline on adipose tissue lipid metabolism is similar to that of adrenaline. Noradrenaline infusion leads to increased ATBF, which results in increased efflux of NEFA and glycerol from adipose tissue, indicating increased activity of HSL (Kurpad *et al.* 1994). It would appear that with catecholamines ATBF is at least as important a regulator of lipid metabolism as HSL and LPL.

The other counter-regulatory hormone cortisol also has profound effects on fuel metabolism during sepsis and trauma. Its effects on protein and carbohydrate metabolism are well documented, but its effects on lipid metabolism are less well understood. Hypercortisolaemia increases the arterial concentration of NEFA and their whole-body turnover. However, arterio-venous studies have shown that hypercortisolaemia reduces the efflux of NEFA from some adipose tissue depots, with associated reduction in HSL rate of action in this depot (Samra *et al.* 1998a). The increased arterial concentration of NEFA can be explained on the basis of increased LPL rate of action as the plasma TAG concentrations are reduced. The other possible effects could be through site-specific actions of cortisol.

In most cases sepsis and trauma can increase lipolysis, as observed by elevated arterial concentrations of NEFA and their increased rate of turnover (Shaw & Wolfe, 1987; Fellander *et al.* 1994). In the event of very severe assault adipose tissue perfusion may be impaired, which may lead to a lower arterial concentration of NEFA and their reduced turnover. One of the major differences between sepsis and trauma appears to be sepsis-induced hypertriacylglycerolaemia. This effect of sepsis appears to be mediated partially through the liver, by selective partitioning of NEFA into TAG and increased *de novo* synthesis of TAG. In addition, the peripheral clearance of TAG is reduced through reduction in LPL levels (Feingold *et al.* 1992).

Future advances in lipid metabolism

In recent years, it has been shown that cytokines such as tumour necrosis factor, leptin and plasminogen activator inhibitor-1 produced by adipose tissue act in an endocrine and paracrine fashion (Fig. 3). These cytokines have profound physiological and pathophysiological effects. They have significant interactions with lipid metabolism in adipose tissue, which in turn implies that adipose tissue may be able to autoregulate its own lipid metabolism. *In vivo* studies have shown that when intralipid is infused into healthy human subjects there is a reduction in the rate of action of HSL, accompanied by rapid fall in the trans-capillary efflux of NEFA, in the absence of any change in plasma insulin concentration (Samra *et al.* 1998b). More importantly, recent studies into adipose tissue peroxisome proliferator-activated receptors have highlighted the role of substrates in molecular mechanisms that may regulate cell growth and apoptosis. The variability in expression of these factors in different tissues not only reflects the cross-linking of metabolism in different tissues, but also provides an insight into the complexity of the control of the regulation of metabolism.

In order to optimally treat conditions such as obesity, cachexia, syndrome X, trauma and sepsis, first we must understand the altered lipid metabolism which appears to be a major component of these phenomena. Over the next few years we are likely to develop a better insight into these complex problems through our understanding of the effects and interactions of various cytokines with lipid metabolism in adipose tissue.

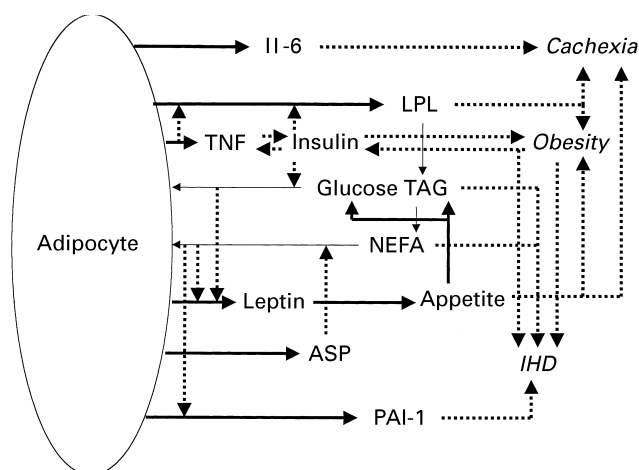


Fig. 3. The regulation of adipose tissue lipid metabolism can be controlled not only by different substrates, but also by numerous adipokines. Adipokines autoregulate adipose tissue lipid metabolism, but also provide mechanisms for the integration of whole-body lipid metabolism. IL-6; interleukine 6; TNF, tumour necrosis factor; ASP, acylation-stimulating protein; PAI-1, plasminogen activator inhibitor-1; NEFA, non-esterified fatty acids; TAG, triacylglycerols; LPL, lipoprotein lipase; —> factor produced by a definite effector mechanism; - - -> possible effector mechanism. Disease processes are shown in italics.

Conclusions

I have predominantly discussed *in vivo* lipid metabolism in human adipose tissue. *In vitro* studies may show a number of potential different regulators of lipid metabolism, but their significance *in vivo* may be lost because of physiological factors which can also affect lipid metabolism. In the last decade there has been rapid growth in our understanding of the regulation of lipid metabolism in adipose tissue during feeding and fasting, and the effect of different hormones. In reality, this progress was probably the tip of the iceberg, as now we enter an exciting phase of deciphering the role of different cytokines in the regulation of lipid metabolism in adipose tissue during different pathophysiological conditions.

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