review

In vivo regulation of lipolysis in humans

Simon W. Coppack, Michael D. Jensen, and John M. Miles[1]

Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905 and University College London Medical School, Whittington Hospital, London N19 5NF, UK

Abstract Fatty acids are important oxidative fuel for liver, kidney, skeletal muscle, and myocardium. There has been much interest in the role of fatty acids in the pathogenesis of non-insulin-dependent diabetes because they compete with glucose for oxygen and inhibit whole body glucose disposal via the 'Randle cycle.' Control of lipolysis in adipose tissue determines systemic fatty acid supply. A wide range of hormones and other substances have been recognized as regulators of lipolysis, but insulin and catecholamines appear to be the most important. The regulation of lipolysis, in most circumstances, provides a supply of lipid fuel exceeding the rate of lipid oxidation, requiring reesterification to triglyceride of surplus circulating free fatty acids. Thus, free fatty acid supply is usually not matched to the demand for lipid oxidation, and there is no known mechanism for accurately sensing such demand. This lax regulation may be disadvantageous in conditions such as aging, stress, obesity, and diabetes, where the antilipolytic effect of insulin is impaired and lipolysis is therefore increased. In these conditions, the surfeit of fatty acid may impair glucoregulation. In addition, the excess lipolysis may induce hypertriglyceridemia (via increased very low density lipoprotein production) and thus contribute to atherogenesis. Considerable additional research is needed in order to fully understand both normal lipolytic regulation and the abnormalities of lipolysis which accompany pathological conditions.—**Coppack, S. W., M. D. Jensen, and J. M. Miles.** In vivo regulation of lipolysis in humans. *J. Lipid Res.* 1994. **35:** 177–193.

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INTRODUCTION: THE RANGE OF LIPOLYTIC PROCESSES

Lipolysis refers to processes in which triglyceride is hydrolyzed, via di- and monoglyceride intermediates, to fatty acids and glycerol (1). Lipolysis occurs both intracellularly and extracellularly in a variety of tissues, and is an essential component of both lipid storage and lipid mobilization. Adipose tissue, liver, and muscle (both cardiac and skeletal) are quantitatively the most important sites of lipolysis; their role is illustrated schematically in **Fig. 1.** As shown, lipolysis occurs *a)* in adipocytes (where the majority of lipolysis occurs), releasing fatty acids into the circulation, *b)* in other cells such as those in liver and muscle to provide fatty acids for local oxidation, and *c)* in the intravascular space, using circulating lipids as substrate.

The vast majority (>95%) of the body's triglyceride (TG) is found in adipose tissue stores (1) (10–15 kg in a healthy young adult), with smaller amounts in other tissues (2, 3). Lipolysis occurs within all triglyceride-storing tissues so that lipid can be mobilized for oxidation of nonoxidative processes such as membrane or eicosanoid synthesis. Adipose tissue lipolysis is the major regulator of the body's supply of lipid energy because it controls the release of fatty acids (FA) into the plasma, where they circulate as free fatty acids (FFA) complexed to albumin (4). Most fatty acids reach the tissues that oxidize them via this circulating pool (3), although they may traverse an intracellular TG pool prior to oxidation (see below). The rate-limiting step for mobilization of adipose tissue TG is hydrolysis by hormone-sensitive lipase (HSL, EC 3.1.1.3), so named because of its responsiveness to insulin and catecholamines (5). Adipose tissue HSL is thus the proximal and determining enzyme for whole-body lipid fuel availability. In the post-absorptive state most detectable lipolysis is that mediated by adipose tissue HSL.

The intracellular TG in liver and muscle (both skeletal and myocardial) has a metabolic importance (3, 6) out of proportion to its small mass (0.2–0.5 kg in a healthy young adult). These stores serve as a buffer that compensates for short-term differences in FFA supply and lipid oxidative demand. Intracellular liver and muscle TG turns over quickly compared to adipose tissue TG (7), providing these tissues with an assured supply of FA even when plasma FFA concentrations reach low levels, as they do during the postprandial period (8). Unfortunately, TG

Abbreviations: FA, fatty acid; FFA, free fatty acid; TG, triglyceride; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; VLDL, very low density lipoprotein; HDL, high density lipoprotein; R_a, systemic appearance rate; HPLC, high performance liquid chromatography; A-V, arteriovenous; GC–MS, gas chromatography–mass spectrometry; LBM, lean body mass; NIDDM, non-insulin-dependent diabetes mellitus.

[1]Address correspondence to Dr. Miles at: St. Luke's Hospital, 44th and Wornell, Kansas City, MO 64111.

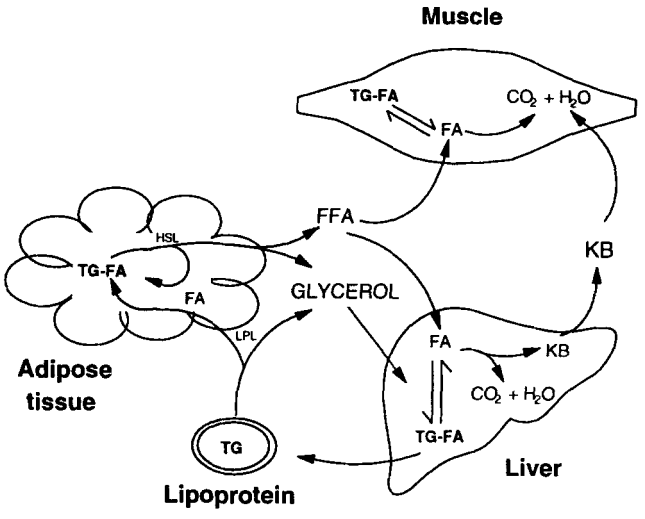

Fig. 1. Simplified scheme of lipid metabolism showing fatty acids in triglyceride (TG-FA) in various tissues. Two major lipolytic enzymes, hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL), act on intra-adipocyte and circulating lipoprotein triglyceride (TG), respectively. Other lipolytic processes occur in muscle (here used as an example of a lipid-oxidizing tissue) and liver. The action of HSL action causes the release of glycerol and free fatty acids (FFA) into the systemic circulation, but some of the fatty acids (FA) may also be reesterified within adipose tissue. LPL action likewise causes the release of glycerol into the circulation but most of the FA from its action on circulating TG are taken up into the adipose tissue. FFA are taken up by muscle and liver for either direct oxidation, partial oxidation to ketone bodies, or reesterification to TG-FA.

metabolism within liver and muscle is difficult to study, and relatively little is known about regulation of intracellular lipolysis within these tissues (3, 7, 9, 10). It is known that fatty acids of muscle TG are derived from both circulating lipoprotein-triglyceride (lipoprotein-TG) and FFA (11, 12), and hence at least partially from adipose tissue lipolysis. Increased muscle TG content has been reported in insulin-resistant states, including non-insulin-dependent diabetes (13). Increased muscle TG has also been reported to result from exercise training (14), however, which is associated with improved rather than impaired insulin action. A recent study was unable to confirm an increase in muscle TG in response to exercise training (15). Thus, it is not clear whether increased muscle TG content results in insulin resistance.

Lipolysis of circulating lipoprotein-TG occurs in the capillary lumen prior to cellular uptake of fatty acids. The fatty acids transported into the cell as a result of this process may then either be reesterified into TG (their predominant fate in adipose tissue) or immediately oxidized (more likely in skeletal muscle or myocardium). This extracellular lipolysis is controlled by lipoprotein lipase (LPL, EC 3.1.1.34), an enzyme bound to the luminal side of the capillary endothelium and activated by circulating apolipoprotein C-II and inhibited by apolipoprotein C-III (16, 17). LPL is usually studied in vitro (17); relatively little in vivo data are available. LPL activity is greatest in adipose tissue (16, 17) where it is regulated reciprocally to HSL (5); adipose tissue LPL is most active postprandially (18) when fatty acids from lipoprotein-TG are taken up for storage (8), and its activity decreases when there is a net flux of fatty acids out of adipose tissue, such as is the case in fasting (19). Lipolysis of circulating TG contributes significantly to plasma FFA postprandially in humans (8, 20). A variety of other lipases (e.g., hepatic lipases) also participate in the clearance of circulating triglyceride (for a more detailed discussion see ref. 21).

The term "lipolysis" thus describes several related processes which may not always be distinguishable in vivo with current methods of measurement. With this limitation, we shall use the term "lipolysis" to refer specifically to HSL-mediated lipolysis except where otherwise indicated.

LIPOLYSIS AND LIPID FUEL AVAILABILITY

Averaged over the 24 h of the day, long chain FA (and their partial oxidation products, ketone bodies) are a major fuel for humans on typical Western diets (22, 23). FA comprise 30–70% of energy supply depending on diet (23) and energy balance (12, 24), being most important in conditions where energy expenditure exceeds intake. Resting muscle, heart, liver, and renal cortex use FA as their primary fuel (2, 7, 11, 25, 26).

Lipolysis must be regulated to ensure an adequate supply of lipid fuel for these tissues (11, 12). The utilization of FA and carbohydrates is balanced through a competitive system of enzyme regulation such that an increased supply of FA inhibits the utilization of glucose (27–29), as originally proposed in the "alternative fuel" hypothesis of Randle et al. (30). If fat oxidation is insufficient, carbohydrate depletion manifesting as hypoglycemia can occur (22, 24, 31, 32). Inhibition of lipolysis accounts for much (almost 50%) of the insulin-induced increase in glucose clearance by skeletal muscle, by reducing the supply of FFA that otherwise compete with glucose as the oxidative fuel in muscle (33, 34). Pharmacological augmentation of FFA availability results in increased lipid oxidation with a reciprocal decrease in glucose uptake and oxidation (27, 35, 36). In addition to its effect on peripheral glucose uptake, increased FFA and glycerol availability stimulates gluconeogenesis (27, 37–39). Consequences of excess FA mobilization in pathological states thus may include impaired glucose disposal and increased hepatic glucose output (38, 40).

In post-absorptive situations, FFA turnover exceeds total body lipid oxidation (41–43). Surplus FFA can be reesterified in a variety of tissues; a major site of reesterification is the liver, where FFA are incorporated into very low density lipoprotein-TG (VLDL-TG) (2, 6, 44, 45).

Thus, excess lipid mobilization and FFA flux may be a major factor in increased hepatic VLDL-TG secretion (44, 46), especially when accompanied by insulin resistance and hyperinsulinemia. Although fasting triglyceride concentrations may be only modestly elevated when VLDL-TG secretion is increased (47), this increased VLDL-TG secretion is accompanied by exaggerated chylomicronemia after an oral fat load, as chylomicron TG and VLDL-TG compete for LPL (48). Circulating VLDL-TG is cleared by LPL which converts triglyceride-rich lipoproteins to remnant particles (49). Either increased synthesis or decreased clearance (or both) lead to an increased concentration of triglyceride-rich lipoproteins (44), which in turn is associated with a decrease in high density lipoprotein-cholesterol (HDL-cholesterol) (50–53). High VLDL-TG concentrations are associated with atherosclerosis (47, 51, 54, 55). The mechanisms for this association are uncertain; putative links include the atherogenicity of usual VLDL or VLDL-remnant particles (56–58), the production of abnormal remnant particles (59, 60), and the depletion of HDL (52, 61). Much remains to be learned about the relationship between atherosclerosis and abnormalities of lipolysis, but it has been postulated that excess FFA flux has a proximal role in the atherogenic lipoprotein profile and increased vascular disease seen in obesity and NIDDM (54, 62).

METHODS OF INVESTIGATION OF LIPOLYSIS IN VIVO

A variety of methods are available for the study of lipolysis in humans, each having advantages and drawbacks (**Table 1**). There is a large literature on in vitro studies of adipose tissue lipolysis. The in vitro approach allows the investigator to control a maximum number of variables while studying lipolytic regulation. However, such studies are undertaken of necessity without input from such factors as adenosine, blood flow, and local sympathetic innervation (see Tables 1 and 2). Moreover, the absence of albumin in many incubation media may lead to anomalous results (63, 64). In fact, a systematic attempt to compare in vitro and in vivo lipolysis yielded a significant negative correlation between the two (64).

TABLE 1. Methods of study of lipolysis

(a) In vitro
(b) In vivo
1) Systemic
Plasma FFA and glycerol concentrations
Infusion of radioactive or stable isotopes
2) Regional/local
Arteriovenous differences
Microdialysis
3) Combinations of the above techniques

The investigation of lipolysis in vivo can be undertaken either by measurement of systemic lipolytic rates or the study of individual tissue depots. To understand human lipolysis, human studies are preferable because of the well-recognized species differences in adipose tissue (65). Differences in diet may contribute to these differences, as diet affects lipolysis (66) and humans and laboratory animals eat different foods.

Systemic measurement of lipolysis

Although changes in FFA and/or glycerol concentrations in the general circulation may reflect qualitative changes in adipose tissue lipolysis, they should not be regarded as a quantitative representation of lipolytic activity because of the nonlinear relationship between turnover and concentration (67, 68) and because FFA clearance may differ in conditions such as exercise (69), diabetes (70), and obesity (71). Measurements of systemic appearance rate (R_a) of FFA or of glycerol by isotope tracer methods are superior and have long been used to measure the rate of adipose tissue lipolysis (72). Tracer techniques have relatively few limitations (73), as they are able to measure R_a accurately under both steady state and non-steady state conditions (67, 74), provided that appropriate methodology such as gas chromatography–mass spectroscopy (75) or HPLC (67) is used and that peripheral venous tracer infusion is combined with sampling of arterial or arterialized venous blood (75, 76). In some situations, lipolysis may yield products that are metabolized without reaching the systemic circulation, and therefore may not be detected by the tracer. This occurs, for example, when the products of splanchnic adipose tissue lipolysis are released into the portal vein and cleared by the liver before they can mix fully with the tracer.

Theoretical limitations exist for both fatty acid and glycerol tracers. Because there is little glycerol kinase activity in adipose tissue (77, 78) and glycerol produced from lipolysis is quantitatively released into the circulation, glycerol R_a should accurately reflect lipolysis. However, uptake of glycerol has been shown to occur in human forearm tissue (79–81), raising the possibility that glycerol, to some extent, is metabolized locally in tissues before it is released into the systemic circulation. Furthermore, although HSL activity in adipose tissue releases glycerol, so may the activity of other lipases, such as lipoprotein lipase (LPL), so that glycerol R_a becomes a composite estimate of the two processes (8). The importance of animal data suggesting that little glycerol is released from LPL action (82) is uncertain. If partial lipolysis of triglyceride to di- and monoglycerides were to occur (83), no glycerol would be released and a fatty acid tracer would be more useful (84, 85).

A more important advantage of measuring the R_a of a fatty acid such as palmitate is that it is more relevant to

metabolic fuel supply. Although there are slight regional differences in the metabolism of different fatty acids (86, 87), the kinetics of the long chain fatty acids are sufficiently similar (88) that the R_a of a single fatty acid such as palmitate can be used as a paradigm for total FFA flux (89). The issue of how best to standardize R_a to facilitate comparison between individuals of differing body composition is discussed below.

Previous in vitro work has shown release of FFA and glycerol from adipocytes at a ratio significantly lower than 3:1, suggesting that FFA may be reesterified locally prior to release from adipocytes (90). If reesterification were to occur at significant rates, rates of measurement of FFA R_a in vivo would not accurately reflect adipose tissue lipolysis. However, Edens, Leibel, and Hirsch (63) have demonstrated that this observation may be an artifact of the experimental conditions. In vivo experiments generally indicate minimal local FFA reesterification (91–93).

Whether a tracer of glycerol or FFA is selected, it is important to recognize the importance of using accurate, specific analytical techniques. If possible, it is best to use a procedure in which the recovery of the tracer (labeled species) and tracee (unlabeled species) are linked, such as gas chromatography–mass spectrometry (GC–MS) or high performance liquid chromatography (HPLC). Earlier methods of analyses generally involved measurement of tracer and tracee in separate assays, and these assays often had inadequate specificity and precision. Early studies reported FFA R_a: glycerol R_a ratios ranging from 1.4:1 (94) to 5.4:1 (95). Such implausible results are likely due to analytical errors; when GC–MS is used, the FFA R_a: glycerol R_a ratio ranges only from 2.6:1 to 3.0:1, in adult humans (91), neonates (92), and animals (93).

Lipid oxidation is often measured by indirect calorimetry concurrently with a determination of systemic lipolysis to determine the fate of mobilized lipid (oxidation versus re-esterification). Indirect calorimetry measures only net lipid oxidation, which may differ from total lipid oxidation (96–98), and it is often difficult to establish which pool of lipid is being oxidized with this technique (3, 7, 99). The proportion of plasma FFA which is directly oxidized is not known, but it is clear that intracellular triglyceride fatty acids are an important preoxidative pool (3, 99).

Study of regional/local lipolysis

Studies of lipolysis in individual tissues can be undertaken by measurement of arteriovenous (A-V) differences or by microdialysis. A-V difference methods have long been applied to forearm muscle (100, 101) and have more recently been used for subcutaneous abdominal adipose tissue (102). This method is based upon the measurement of metabolite concentrations in venous blood draining adipose tissue. Release of both glycerol and FFA from adipose tissue have been reported (8). Although ideally undertaken under steady-state conditions (100, 103), A-V difference measurements are valid in non-steady-state situations (104), presumably because of the high fractional turnover of these substrates. Contamination of the venous effluent by blood draining skin is a minor problem, as skin is relatively inactive as a lipolytic tissue and relies primarily on glycolysis to generate ATP (105, 106). Measurement of A-V differences in circulating triglyceride concentrations permits the distinction of lipid-storing lipolysis (LPL action) from lipid-mobilizing lipolysis (HSL action) and is most useful when combined with a measure of blood flow (8).

Microdialysis involves inserting one or more semipermeable dialysis catheters within a tissue. Fluid is then pumped slowly ($\sim 0.5\ \mu l \cdot min^{-1}$) through the catheters and interstitial tissue concentrations are estimated from the concentration measured in the effluent dialysate fluid. The method requires steady-state conditions for optimal quantitation of interstitial concentrations (107). Although only subcutaneous adipose tissue has been studied, different depots may be examined (108, 109). Contamination of microdialysate with skin interstitial fluid is thought to be of little importance (104, 109). Local pharmacological or physiologic effects of dialyzable drugs or hormones can be investigated by adding the drug or hormone to the dialysate (110, 111). With respect to lipolysis, interstitial glycerol concentrations can be assayed by microdialysis (108, 110, 111). The technique may not be applicable to the study of FFA metabolism, because FFA are not dialyzable using standard dialysis membranes (4). Glycerol and lactate concentrations in adipose tissue interstitial fluid are reported to be the same as those in the adipose tissue venous effluent (104). In situations where blood flow or capillary permeability changes (112), a change in local glycerol concentration may not be a quantitative reflection of glycerol release/lipolysis rates (111). Caution is warranted when studying individual adipose depots, because heterogeneity between various adipose tissue depots makes it difficult to extrapolate the results to whole body lipolysis (113–117).

METHODOLOGY FOR THE STUDY OF LIPOLYSIS

The choice of methodology ultimately depends upon the question being addressed. Measurement of whole-body FFA R_a is best for study of the overall supply of lipid fuel for oxidation, and FFA R_a correlates well with whole-body fatty acid oxidation rates as measured by indirect calorimetry (64). A combination of glycerol and FFA tracers has been used for measuring re-esterification (42, 118–121), but this approach requires assumptions about the individual methods which may be tenuous (see above). Studies of local depots are most useful for investi-

gation of lipolytic regulation (physiological or pharmacological) in adipose tissue per se. Combinations of local and systemic methods can be used to characterize regional differences in adipose tissue lipolysis (115, 117).

Studies of the regulation of lipolysis are sometimes confounded by interactions between regulatory mechanisms. For example, administration of a hormone that raises plasma glucose (e.g., glucagon) may inhibit lipolysis indirectly via an increase in plasma insulin concentrations due to hyperglycemia. Because of this, it is helpful to conduct studies under conditions in which as many variables as possible are controlled. A convenient means for accomplishing this end is the so-called 'pancreatic clamp' (122–124) in which somatostatin is infused to block secretion of endogenous hormones, and then those hormones are infused continuously in order to maintain their plasma concentration constant. This approach has been used with considerable success in the study of the regulation of glucose metabolism (125–127). The 'pancreatic clamp' has the advantage that the insulin dose–response curve may be examined in the range of very low insulin concentrations (123), even in obese subjects; however, breakthrough secretion of insulin despite somatostatin infusion is a technical problem in such studies. Moreover, it may be necessary to reproduce the pulsatile pattern of insulin and growth hormone secretion in order to optimize the clamp technique. The pancreatic clamp cannot control all variables that may modulate lipolysis; for example, there may be local changes in sympathetic nervous system activity, adenosine and/or blood flow (see Tables 1 and 2). Each of these factors should be considered when designing experiments and interpreting their results.

Problems in interpretation of changes in lipolytic rates in response to regulators of lipolysis such as insulin or catecholamines occur when basal rates are different between groups. For example, the obese have higher basal rates of lipolysis but show less increase, both relative and absolute, in response to lipolytic stimuli (see below).

MECHANISMS OF REGULATION OF LIPOLYSIS

Inhibitors of lipolysis

Numerous factors may act, either individually or in concert, as significant inhibitors of lipolysis in a variety of conditions (**Table 2**).

Insulin is by far the most potent antilipolytic hormone and has been extensively studied (35, 41, 114, 123, 128). There is general agreement that in normal subjects half-maximal suppression of whole-body lipolysis occurs at insulin concentrations well below those needed to directly stimulate glucose uptake by skeletal muscle (41). Recent work suggests that lipolysis in normal subjects is exquisitely sensitive to insulin with a half-maximal effect (ED_{50}) occurring at a concentration of 12 pmol·l^{-1} (**Fig. 2**) (123). Higher concentrations of insulin (200–300 pmol·l^{-1}) are able to reduce the systemic R_a (123) and local output (128) of FFA to nearly zero. In contrast, the R_a of glycerol cannot be fully inhibited by insulin (129), possibly because insulin stimulates LPL (16) and the action of adipose tissue LPL on circulating lipoprotein-TG releases glycerol into the circulation (8) somewhat out of proportion to FFA (82). HSL action, as measured by local glycerol release (taking LPL action into account), decreases within 15 min after the start of an insulin infusion (128).

TABLE 2. Inhibitors of lipolysis

Hormonal	
Insulin	Important regulator; see text
α_2 Adrenergic agonists	Some effects, less important than β-adrenoreceptor effects (143); importance may vary with gender and adipose tissue site (130, 143); α-blockers being considered as weight-reducing agents (144)
Somatostatin	Some effect in vitro (145), physiological relevance uncertain
IGF-1	Lowers FFA concentrations in vivo (146), physiological relevance uncertain
Substrate	
Adenosine	Regulator in vitro (130), interstitial adenosine concentrations are in a range shown to affect lipolysis (131); see text
FFA	Effects in vitro (140), not yet confirmed in vivo
Ketone bodies	Significant effects in vivo (132), has potential physiological importance; see text
Glucose	No effect according to most (147, 148, 149) but not all (124) in vivo studies
Lactate	Effects in vitro (150) and dogs (151), not confirmed in man when other hormone concentrations clamped (152)
Glutamine	Changes plasma FFA and glycerol concentrations (153), but not when other hormone concentrations clamped (154)
Prostaglandins	PGE_2 potent antilipolytic agent in vitro (155, 156); physiological importance uncertain
Physical	
Reesterification	In vitro studies suggest important mechanism (112), in vivo studies suggest unimportant during exercise (42, 135), reesterification within adipose tissue may be part of mechanism of action of insulin (128); see text
Adipose tissue blood flow	Dog studies suggest major regulator (137), importance in man less certain; see text
Pharmacological	
Acipimox/nicotinamide	Basis of antilipid therapeutic effect (157)
β-blockers	Regulate via β_1 effects (111, 158), antilipolytic action may contribute to weight gain with propranolol (159)
Palmoxirate/etomoxir	An insulin secretagogue in animals when given intravenously (160)

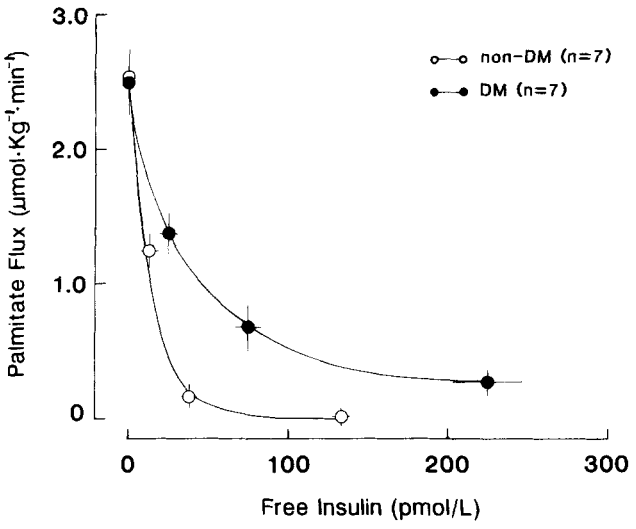

Fig. 2. Steady-state palmitate flux plotted versus plasma free insulin concentrations for diabetic (●) and nondiabetic (○) subjects.

Adenosine is present in adipose tissue and has been shown to inhibit HSL (via inhibition of cyclic AMP in vitro) (130). Because adenosine concentrations are extremely low in adipose tissue and because partitioning of adenosine between tissue and blood occurs due to the endothelial barrier, it is difficult to investigate the in vivo importance of adenosine as a local modulator of lipolysis. However, the use of microdialysis shows great promise in this regard (131). Additional research is needed to determine whether tissue adenosine concentration serves to modulate the lipolytic response to primary effectors such as insulin and catecholamines.

The ketone bodies acetoacetate and β-hydroxybutycate are potent inhibitors of lipolysis, decreasing FFA R_a by ~75% from basal rates in postabsorptive subjects (132). These effects are demonstrable at plasma ketone body concentrations that occur after strenuous exercise, after short-term fasting, and in uncontrolled diabetes, indicating that ketone bodies may be important modulators of lipolysis in these conditions. The exact biochemical mechanism by which ketone bodies exert this effect is not clear, but the ketone bodies appear to be an important respiratory fuel for adipose tissue (133). It is possible that the ketone bodies may be important modulators of the lipolytic response to insulin, catecholamines, and growth hormone.

Reesterification of fatty acids back to triglyceride within adipocytes may regulate the appearance of FFA from adipose tissue (42, 43, 112) and hence the metabolic sequelae of lipolysis. Whole-body studies with isotopes show that in the fasting state, the R_a of FFA is less than three times that of glycerol (42, 43), suggesting that reesterification is occurring within lipolytic tissue (for fuller discussion, see refs. 43 and 134). However, the site of such reesterification, or even whether it occurs, cannot be conclusively established by whole-body tracer methods (43). For example, if hepatic uptake of FFA derived from mesenteric lipolysis is out of proportion to hepatic glycerol uptake, this could account for a FFA:glycerol turnover ratio of less than 3:1 without actual reesterification in adipose tissue. Fasting A-V difference measurements suggest that in a single adipose depot 5–20% of FFA is reesterified within the adipose depot (134–136), a value compatible with the "intracellular reesterification" ratio of Wolfe et al. (42, 43). However, much of this "reesterification" may in fact reflect uptake of FA and release of glycerol from the action of LPL on circulating TG. Postprandially and during euglycemic hyperinsulinemia, this local reesterification ratio approaches 100% (128, 134). Insulin's inhibition of HSL has a greater impact (approximately threefold) upon FFA release than does its effect on reesterification (8, 128). Reesterification in in vitro studies is difficult to interpret because such reesterification is related to experimental conditions such as tissue preparation and availability of albumin in the medium (63).

It has been proposed that blood flow is a major regulator of delivery of FFA to the circulation (137, 138). Low blood flow could potentially result in saturation of the limited number of high affinity FFA-binding sites on albumin (4) and result in an accumulation of FFA within adipose tissue (137). High local FFA concentrations could promote increased reesterification by mass action (139) and might also directly inhibit HSL activity, as suggested by some (140), but not all (63) studies in vitro. The blood flow hypothesis is supported by work in dogs (141) and may explain discrepancies between in vivo FFA R_a and in vitro results (112). However, FFA concentrations in plasma are frequently >2 mmol·l^{-1} (135, 142) thus exceeding the capacity of the high affinity FFA-binding sites on albumin (4); this implies that systemic lipolysis continues even when the high-affinity sites are saturated. During exercise there is no evidence of an increase in reesterification ratios within human adipose tissue (42, 135), although exercise of very high intensity has not been studied. Thus it is not clear whether changes in blood flow are of importance in the regulation of human lipolysis.

Promoters of lipolysis (Table 3)

Catecholamines are powerful regulators of lipolysis. In both in vivo and in vitro studies, stimulation of β_1-adrenoreceptors in adipose tissue (from at least some sites) dominates over α_2-adrenoreceptor inhibition of lipolysis (161, 162). Catecholamines may reach adipose tissue via the general circulation or via the rich sympathetic innervation (163), which makes accurate assessment of their role in lipolytic regulation difficult. Systemic infusion of epinephrine increases palmitate R_a (161, 164) with the threshold for stimulation occurring at plasma concentrations of ~440 pmol·l^{-1} (161). Microdialysis studies

TABLE 3. Stimulators of lipolysis

Hormonal	
β Adrenergic agonists	Important regulators, see text
Growth hormone	Potentially important regulator, see text
Glucocorticoids	Potentially important regulator, see text
Glucagon	Inconsistent effects in vitro and in vivo, stimulatory effects not found when other hormone concentrations clamped (179)
Thyroxine	Thyrotoxicosis stimulates lipolysis (180)
Sex steroid hormones	Effects in animals (181), of limited importance in healthy women (182)
Tumor necrosis factor α	Acute lipolytic effect in normal volunteers (183)
Substrate	
Prostaglandins	PGI_2 powerful lipolytic agent in vitro (156, 184)
Pharmacological	
Caffeine/Thioxanthines	Mild effect (185), not used therapeutically
Heparin	Releases LPL into the circulation (17)
Adrenergic agents	Conventional β-agonists not used therapeutically, β_3-agonists being studied (186)

have shown stimulation of lipolysis by very low local norepinephrine concentrations (~ 10 pmol·l^{-1}) (110), and this finding is supported by in vivo studies in dogs (165). Short-term systemic β-blockade reduces FFA R_a, but the effect appears to wane with longer-term administration (158). Several adrenergic receptor systems involved in lipolysis show tachyphylaxis (110). The relative importance of local synaptic versus systemic delivery of catecholamines is not fully established, but it is apparent that local norepinephrine from sympathetic nerve terminals within adipose tissue is a major lipolytic promoter (163). Results from microdialysis studies suggest that α-adrenoreceptors predominate in the regulation of adipose tissue lipolysis at rest and that β-adrenoreceptors assume a more important role during exercise (111). There is considerable regional heterogeneity in the sensitivity of adipose tissue depots to adrenergic agents (113, 166, 167).

The discovery of the β_3 receptor in brown adipose tissue (168) is an exciting development in the study of adipose tissue lipolysis and energy metabolism. Recently, Krief et al. (169) demonstrated mRNAs for both β_3 receptor and mitochondrial uncoupling protein scattered in an ubiquitous fashion throughout white adipose tissue of humans undergoing elective surgery. In this report, β_3 receptor mRNA levels were particularly high in perirenal and omental fat, and β_3 activity was found in isolated white adipocytes that did not possess mRNA for the uncoupling protein. These observations document unequivocally the presence of brown adipose tissue in adult humans and have significant implications in the study of thermogenesis and obesity. Whether β_3 stimulation results in significant systemic delivery of FFA and glycerol is uncertain and will require in vivo studies with highly selective β_3 agonists.

Growth hormone stimulates lipolysis in vitro (170) and at physiological concentrations in vivo (171), but its relative rank in the hierarchy of lipolytic regulators is not yet entirely clear. The physiological importance of growth hormone has been most clearly demonstrated in relation to nocturnal FFA availability (171, 172) and it may be important in the response to stress (173, 174).

Some studies have shown that either endogenous (175) or exogenous (173) glucocorticoid excess inhibits lipolysis, whereas others found no such effect (176, 177). However, when compensatory hyperinsulinemia during cortisol infusion was prevented by a pancreatic clamp, cortisol infusion resulted in a 60% increase in palmitate R_a (122). Thus endogenous cortisol may be an important lipolysis-stimulating hormone in conditions of limited insulin secretion such as stress (178) or diabetes mellitus, and could account via this mechanism for some of the glucose intolerance/insulin resistance of both conditions.

INTEGRATION OF LIPOLYTIC REGULATION IN NORMAL CONDITIONS

In normal subjects, regulation of lipolysis within adipose tissue in response to feeding, fasting, and exercise appears based upon interactions among several of the regulators discussed above, principally plasma insulin concentration and adrenergic modulation.

Post-absorptive and post-prandial states

In post-absorptive states such as an overnight fast, there is marked day to day variability in FFA concentrations and FFA turnover in normal subjects [coefficient of variation ~30% (123)]. This relatively inexact regulation matters little, as the R_a FFA exceeds the oxidative demand for lipid by about 100% (35, 41) and excess FFA are recycled by reesterification in tissues such as muscle and liver (see above) (2, 6). In this respect, regulation of lipolysis is different from that of glucoregulation, in which plasma glucose concentration is tightly maintained (187) to avoid the extremes of diabetes mellitus and neuroglycopenia. The day to day variability of FFA R_a is substantially reduced by the pancreatic clamp procedure (123), suggesting that some of the variability is due to

small changes in plasma insulin concentrations (see above). By this mechanism, glucose and other insulin secretogogues indirectly regulate the basal rate of lipolysis.

In general terms, it is unclear what "sets" the rate of adipose tissue lipolysis. It is apparent that lipolysis is controlled largely by sympathetic activity and insulin concentrations. However, whether FFA availability exerts feedback on these processes, in a fashion analogous to glucoregulation, is not clear. FFA and ketone bodies are capable of stimulating insulin secretion (188, 189), but rank relatively low in the hierarchy of insulin secretogogues. Certainly, there is no evidence for a receptor system measuring FFA availability and altering sympathetic drive accordingly. Thus, it seems unlikely that there is a primary feedback mechanism that tightly matches adipose tissue lipolysis to the demand for lipid fuel on a minute to minute basis; the availability of triglyceride stores in tissues such as liver and skeletal muscle and a recycling system (i.e., reesterification) for surfeit FFA mobilized into plasma make such a mechanism unnecessary.

The changes in lipolysis that occur in response to eating involve several of the mechanisms discussed above. Compared to the post-absorptive state, a transformation from lipid mobilization to lipid deposition occurs with an increase in carbohydrate oxidation (190). These changes are orchestrated primarily by the postprandial insulin response. HSL activity (8, 134) and FFA R_a (20) are reduced while LPL is stimulated (8, 134) so that adipose tissue changes from net lipid export (of FFA) to net lipid import (of lipoprotein-TGFA).

Starvation and exercise

Starvation and exercise represent the two main physiological situations of increased lipolysis. After an overnight fast, lipid oxidation can account for >70% of total body energy expenditure (191) and FFA R_a exceeds lipid oxidation (35, 41, 43, 99). With fasting of longer duration there is progressive stimulation of lipolysis and elevation of FFA which favors sparing of carbohydrate (30). A progressively greater proportion of total body fuel demand is met by lipid oxidation (192), with the R_a of glycerol and FFA increasing in proportion (118, 164). During a fast of several days, the rate of lipolysis continues to exceed the rate of lipid oxidation. However, the metabolic cost of reesterifying the unoxidized FFA remains low (192). In starvation states, decreasing plasma insulin concentrations allow derepression of HSL and thus increased lipolysis. Increasing cortisol and growth hormone concentrations may also play a role. Some workers (164, 193), but not all (194), found a decrease in the responsiveness of lipolysis to insulin and other antilipolytic agents with prolonged fasting in normal subjects. It is uncertain how such a change in insulin sensitivity is mediated, although increasing sympathetic activity or responsiveness may play a part (158, 164).

Sustained exercise of moderate intensity is characterized by marked increases in lipolysis with a several (4- to 10-) fold increase in FFA R_a in response to increased energy requirements (11, 22, 69). Elite athletes are able to mobilize sufficient fatty acids in response to exercise (12). For that matter, relatively unathletic subjects show an appropriate increase in lipolysis, sufficient to match the increase in fat oxidation required by low intensity exercise (195).

Muscle TG stores are depleted during prolonged submaximal exercise (15, 24), suggesting that circulating FFA are insufficient to meet energy needs of muscle in this situation. In lower level exercise R_a FFA approximates oxidative demand (42, 195). This does not mean that turnover of muscle TG is not occurring in this situation (or indeed even at rest); a significant fraction of FFA entering muscle may traverse the intracellular TG pool prior to oxidation (3, 99). Repletion of muscle TG stores after a marathon requires longer than 7 days (196). Histologic studies show that Type I muscle fibers contain approximately 3 times more TG than Type II fibers (197); this coincides with the greater prevalence of mitochondria in Type I fibers (198). The exercise-related increase in lipolysis may be due to decreased insulin concentrations (42, 135, 195) and/or increasing adrenergic stimulation (111). Much of the lipolytic response to exercise can be blocked by sympatholytic agents (111, 199), suggesting that catecholamines play a dominant role in the stimulation of adipose tissue lipolysis during exercise.

ABNORMALLY REGULATED LIPOLYSIS

Clinically important problems result from failure to limit lipolysis. The consequences of unrestrained lipolysis are most dramatic in diabetic ketoacidosis (142), whereas lesser degrees of excess lipolysis may worsen glucose tolerance and promote hypertriglyceridemia (44, 200).

Stress

In stress conditions, of which trauma, burns, surgery, and malignancy are the most studied, lipolysis increases despite hyperinsulinemia (120, 201). Increased sympathetic drive and stress hormones presumably antagonize the antilipolytic action of insulin (119), while insulin secretion is restrained by α-adrenergic effects (202). In stress conditions lipolysis is stimulated to an inappropriate degree, with reesterification increasing to "mop up" the excess FFA liberated (120). It is possible that down-regulation of lipolysis occurs due to sustained catecholamine excess in such conditions as injury or sepsis; such down-regulation would have a moderating influence on the increased lipolysis that occurs in these conditions.

Obesity

Obesity is well recognized as a condition associated with increased lipolysis, reflected by increased systemic glycerol and FFA concentrations (203) and R_a (28, 41, 116, 117, 204–207) and by increased concentrations of glycerol (208) and FFA (134) in the venous effluent from adipose tissue. Weight reduction improves at least some of the abnormalities of lipolysis in obesity (207, 209). One problem in quantifying the excess lipolysis of obesity is whether the FFA and glycerol R_a should be expressed in terms of lean body mass (LBM), adipose tissue mass, or total body weight. The expression of these indices can have considerable influence on data interpretation. For example, obese subjects have increased basal rates of lipolysis when FFA R_a is expressed in LBM units but have a reduced basal FFA R_a (and increased sensitivity to the antilipolytic effects of insulin (see below) when expressed in terms of total body fat (205). Since the primary purpose of lipid fuel mobilization is to satisfy energy needs, it is preferable to express FFA and glycerol kinetic data relative to energy expenditure (oxygen consumption) or to LBM [because LBM strongly correlates with total energy expenditure at rest (210)]. In contrast, for comparison of the lipolytic activity of separate fat depots in the same subject, regional fat mass would be the more appropriate denominator (117).

FFA R_a in obese, compared to lean, subjects increases less in response to epinephrine (116, 206), to exercise (195, 205), and to starvation (206). However, the higher resting, post-absorptive FFA turnover (relative to LBM) in obesity may partly account for this, because absolute FFA R_a under all three conditions of stimulated lipolysis are similar in lean and obese subjects.

Resistance to insulin's antilipolytic effect and hyperinsulinemia usually accompany obesity (especially upper body obesity), but the hyperinsulinemia is not sufficient to prevent increases in FFA R_a (28, 116). The cause of insulin resistance in adipose tissue in this situation is unknown. The antilipolytic effect of insulin is reduced in obesity when FFA R_a is expressed in LBM units (116, 204). Similarly, in A-V balance studies, obese subjects show decreased inhibition of HSL and decreased stimulation of LPL postprandially despite hyperinsulinemia (134). Dose–response studies suggest that in obesity per se the insensitivity to insulin may be primarily a change in ED_{50} (204).

Aging is associated with an increased body fat content (118, 211) and in many respects resembles obesity, as there is decreased basal lipolysis rate per unit of adipose tissue (118). With aging, although resistance to insulin's antilipolytic effect is present, the dominant effect on lipolysis during exercise may be decreased sensitivity to the lipolytic action of catecholamines (118).

Compared to lower body obesity, upper body obesity is associated with higher fasting FFA R_a but reduced responsiveness to epinephrine infusion (116) and to exercise (195). These differences in FFA metabolism between upper body and lower body obesity likely reflect regional, as opposed to generalized, differences in adipose tissue lipolysis (115, 117). In vitro studies suggest that there are differences in both the insulin responsiveness (212) and the adrenergic regulation of different adipose depots (113, 166), and that fat cell size influences lipolysis (213). How this adipocyte size differential arises initially is unknown. The development of insulin resistance in large adipocytes may act as a brake on further triglyceride accumulation, and hence protect against continued weight gain (214).

Visceral obesity is especially strongly associated with NIDDM, hyperlipidemia, and hypertension (215, 216). Although the mechanism(s) of the effect is unknown, it has been postulated that omental and mesenteric adipocytes, which show high lipolytic activity in vitro (113, 143), release excess FFA into the portal circulation (216). High intraportal FFA concentrations are hypothesized in turn to stimulate VLDL-TG synthesis (44) and to decrease hepatic insulin clearance (217), resulting in systemic hyperinsulinaemia (216). It is conceivable that pathologic increases in visceral fat mass in individuals who otherwise do not meet standard criteria for obesity (218) may contribute to the development of diabetes and hypertension (214, 215, 219).

On thermodynamic grounds, the increased lipolysis of obesity appears paradoxical. It implies an excess mobilization of fat which would mitigate against obesity, because even if FFA are reesterified, such 'futile cycling' back to triglyceride should consume energy and favor weight loss (220). However, the energy cost of triglyceride-fatty acid cycling is low (42, 192). The value of lipolysis-promoting adrenergic agents (144) is therefore questionable, as such agents have little fundamental impact on energy balance. An exception would be highly selective β_3 adrenoreceptor agonists because of their potential stimulatory effect on energy expenditure in brown adipose tissue (see above) (186).

Diabetes mellitus

Non-insulin-dependent diabetes mellitus (NIDDM) per se resembles obesity (and of course the two conditions often co-exist) in that fasting FFA concentrations are increased and the antilipolytic effect of insulin is diminished in both conditions when the appropriate LBM denominator is used (41, 62, 204, 221). However, abnormalities in FFA clearance as well as lipolysis in NIDDM have been reported (70). The strong association of NIDDM with upper body obesity (see above) (216, 222) makes the relative impact of obesity and diabetes itself on lipolysis difficult to ascertain. Thus, Groop et al. (204) reported similar FFA concentrations and R_a in obese nondiabetic

and obese diabetic subjects. Resistance to insulin's glucoregulatory actions is thus usually accompanied by resistance to insulin's antilipolytic action; however, some individuals have one defect more severely than the other (200, 204, 209, 223–225). There does appear to be some additional decrease in insulin's antilipolytic action in NIDDM compared to weight-matched nondiabetic subjects, possibly a reduction in the maximal response (41, 204). Regardless of the mechanism, even subjects with impaired glucose tolerance or mild NIDDM often have abnormalities of FFA regulation (62, 223). Conventional insulin or sulfonylurea therapy tends to ameliorate, but not normalize, FFA regulation in NIDDM (219, 226, 227). Similarly, plasma triglyceride concentrations may (228, 229) or may not (226) improve but typically remain elevated whereas HDL-cholesterol remains low (55, 226, 228–230) despite hypoglycemic therapy. These lipid abnormalities often antedate overt hyperglycemia in individuals destined to develop diabetes (230).

In poorly controlled IDDM, lipolysis is less sensitive to inhibition by insulin (123), and in ketoacidosis the unopposed action of growth hormone (174), cortisol, and catecholamines markedly increases lipolysis (142, 231). However, ketoacidosis can be reproduced experimentally in the absence of elevated cortisol and growth hormone concentrations (232). In fact, it is not clear that plasma FFA are the immediate precursors for β-oxidation in the liver; it is possible that circulating FFA are taken up and at least partly esterified to TG before undergoing conversion to ketone bodies. Thus liver TG stores may be the immediate substrate for ketogenesis. The considerable temporal delay between the lowering of plasma FFA concentrations after the start of treatment for ketoacidosis and a response in ketone body concentration and R_a would support this interpretation (233).

In IDDM, excess lipolysis frequently accompanies hypoinsulinemia, but adequate insulin therapy usually normalizes plasma FFA and triglycerides (234). Conversely, NIDDM is linked to obesity with an adipose tissue distribution that may account for the observed resistance to the antilipolytic effect of insulin. Thus, in NIDDM these factors promote both excess lipolysis and hepatic reesterification of FFA to TG. The ensuing excess synthesis of VLDL-TG causes hypertriglyceridemia (44, 62, 200, 221), which contributes in turn to a reciprocal lowering of HDL-cholesterol. Even when therapeutic intervention improves glycemic control in NIDDM, these lipoprotein abnormalities usually persist (55) and therefore the risk of atherosclerosis persists. The relative contributions of hyperglycemia per se, of hyperinsulinemia per se, and of dyslipidemia in the atherosclerotic risk observed in obesity/insulin resistance/NIDDM remain uncertain and should be the subject of continued research.

SUMMARY

Lipolytic processes are heterogeneous and complex, and their regulation is poorly understood. Although intracellular lipolysis in skeletal muscle and liver participate in the lipid fuel economy of these tissues, lipolysis in adipose tissue, which is controlled by numerous local and hormonal factors, is the major determinant of systemic lipid fuel availability. Among circulating substances, insulin and catecholamines are probably the dominant regulators, with potentially important roles for growth hormone and ketone bodies. In addition to modulation by circulating hormones and substrates, there are important effects of the sympathetic nervous system, and perhaps adenosine and blood flow as well. Dysregulation of adipose tissue lipolysis occurs in such conditions as obesity and diabetes mellitus, and may contribute to the insulin resistance and hyperlipidemia seen in those conditions. Measurement of lipolysis is an analytical challenge, but newer techniques (including GC–MS, HPLC, abdominal vein cannulation, and microdialysis) are available for its study.

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