

Metabolism of lipids in human white adipocyte

V Large, O Peroni, D Letexier, H Ray, M Beylot

SUMMARY

Adipose tissue is considered as the body's largest storage organ for energy in the form of triacylglycerols, which are mobilized through lipolysis process, to provide fuel to other organs and to deliver substrates to liver for gluconeogenesis (glycerol) and lipoprotein synthesis (free fatty acids). The release of glycerol and free fatty acids from human adipose tissue is mainly dependent on hormone-sensitive lipase which is intensively regulated by hormones and agents, such as insulin (inhibition of lipolysis) and catecholamines (stimulation of lipolysis). A special attention is paid to the recently discovered perilipins which could regulate the activity of the lipase hormone-sensitive. Most of the plasma triacylglycerols are provided by dietary lipids, secreted from the intestine in the form of chylomicron or from the liver in the form of VLDL. Released into circulation as non-esterified fatty acids by lipoprotein lipase, those are taken up by adipose tissue via specific plasma fatty acid transporters (CD36, FATP, FABPpm) and used for triacylglycerol synthesis. A small part of triacylglycerols is synthesized into adipocytes from carbohydrates (lipogenesis) but its regulation is still debated in human. Physiological factors such as dieting/fasting regulate all these metabolic pathways, which are also modified in pathological conditions e.g. obesity.

Key-words: Free fatty acids · Triacylglycerols · Hormone-sensitive lipase · Perilipin · Lipogenesis · Lipolysis.

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RÉSUMÉ

Métabolisme lipidique dans le tissu adipeux blanc humain

Le tissu adipeux humain est l'organe de stockage d'énergie le plus important de l'organisme. Constitué de triacylglycérols mobilisés par la lipolyse et qui représentent un apport énergétique pour les autres organes, le tissu adipeux libère des substrats captés par le foie pour la néoglucogénèse (glycérol), et pour la synthèse des lipoprotéines (acides gras libres). La libération adipocytaire de glycérol et d'acides gras est principalement dépendante de la lipase hormono-sensible dont l'activité est contrôlée principalement par l'insuline et la noradrénaline. Les périlipines semblent également jouer un rôle important dans cette régulation. La majorité des triglycérides plasmatiques proviennent des lipides d'origine alimentaire sous forme de chylomicron dans l'intestin ou de VLDL dans le foie. Libérés par la lipoprotéine lipase dans la circulation sous forme d'acides gras non-esterifiés, ceux-ci sont captés par le tissu adipeux grâce à des transporteurs spécifiques (CD36, FATP, FABPpm) pour la synthèse des triacylglycérols. Une faible partie des triacylglycérols est synthétisée dans les adipocytes à partir des hydrates de carbone (lipogénèse) mais sa régulation est encore mal connue chez l'homme. L'ensemble de ces voies métaboliques est régulé par des facteurs physiologiques dont le principal est l'apport nutritionnel. L'obésité se caractérise souvent par un métabolisme lipidique anormal.

Mots-clés : Acides gras libres · Triacylglycérols · Lipase hormono-sensible · Perilipine · Lipogénèse.

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In quantitative terms, white adipose tissue is the largest energy storage organ of the body (around 10-15 kg in a non-obese young adult which is equal to 135,000 kcal or to the energy of 200 meals). More than 95% of the body's lipids are found in adipose tissue stores as triacylglycerols (TAG) with small amounts in other tissues (liver and muscle) [1]. In normal adult human, only white adipose tissue is present; the brown adipose tissue almost completely disappeared. Less than 0.1% of the body's lipids are in the plasma.

Most of the energy stored comes from ingested TG appearing in the circulation incorporated in chylomicrons. Some FA are also synthesized in the liver and the adipocytes through lipogenesis (de novo fatty acid biosynthesis from non lipid substrates mainly carbohydrates). Between meals, to meet the energy needs of other organs, the TAG stores are mobilized in adipose tissue through the lipolysis process which refers to the hydrolysis of TAG into fatty acids (FA) and glycerol [2]. Leaving the adipocytes, the FA circulate in plasma as free fatty acids (FFA) bound to plasma albumin to be utilized principally by adipocytes (re-esterification into TAG), muscle (oxidation) and liver (oxidation by also for TAG synthesis and VLDL secretion).

Adipose tissue lipid metabolism (TAG storage by and FA release from adipocytes) is highly regulated by hormones (insulin and catecholamines mainly in human [3]) and other factors such as nutritional status (feeding, fasting) and exercise. The integrity of the processes regulating the adipocytes metabolism is essential to maintain the body weight homeostasis and dysregulation of these processes play probably an important role in pathologies such as obesity, insulin resistance, type 2 diabetes.

This review will focus on the regulation of the FA metabolism in human white adipocytes, on the physiological role of this process, and its alteration in obesity.

Triacylglycerol synthesis and storage

Sources of fatty acids

De novo fatty acid synthesis: lipogenesis (Fig 1)

De novo lipogenesis (DNL) is the synthesis of fatty acids molecules from non lipids substrates, mainly carbohydrates. Glucose is the major substrate for DNL. The two sites of DNL are liver and adipocytes.

Hepatic DNL is the most studied. Studies in healthy humans concluded that hepatic DNL is a minor contributor to the fatty acids used for liver TAG synthesis and secretion and is quantitatively minor (~1 g/day) [4-7] compared to the daily amount of TAG available from oral intake (> 80 g/day in western countries). DNL, in liver, can be largely increased (2-4 fold) by high carbohydrate diet (HCHO) [8-12], and is increased in ad libitum fed obese subjects [6, 10] and in hypertriglyceridemic type 2 diabetic patients [13] but still remains minor compared to the oral uptake. New molecules of fatty acids provided by hepatic DNL must be exported incorporated in TG-VLDL before their eventual uptake and storage by adipocytes.

The contribution of adipose tissue to *de novo* fatty acid synthesis is less well defined in human in general and particularly in pathological situations such as diabetes and obesity. It is clear that the key enzymes for fatty acids synthesis, fatty acid synthase (FAS) and acetyl coenzyme A carboxylase 1 (ACC1), are present in human adipose tissue [14-17]. DNL is more active when expressed per gram of tissue, in liver than in adipocytes but it is generally admitted that quantitatively the 1.5 kg of liver and the 10-15 kg of adipose tissues [18] of an healthy adult human produce the same daily amount of fatty acids through lipogenesis (1-2 g/day for each tissue or up to 4 g/day for both).

The regulation of DNL by hormones (insulin, glucagon) and by nutritional conditions (carbohydrates and polyunsaturated fatty acids PUFA) is less well defined in human either in liver or in adipocytes, than in rodent. In rodent, hepatic DNL is very responsive to modification of hormonal and nutritional conditions. Insulin and glucose stimulate hepatic DNL while glucagon and polyunsaturated fatty acids inhibit it. Recent evidence suggest that the regulation of lipogenic genes expression by insulin and PUFA is mediated by sterol responsive element binding protein 1c (SREBP-1c) and in part by LXR ζ (insulin and PUFA) and carbohydrate responsive element binding protein ChREBP (PUFA) whereas the effect for glucose and glucagon could be mediated by ChREBP [19-24]. Much less information are available concerning the regulation of DNL in adipocytes, particularly in human. It is clear that the main transcription factors, SREBP-1c and ChREBP, and the cofactors (Sp1 and Nfy) are present in human adipose tissue [14]. It is known that insulin increases FAS expression and activity in human

Abbreviations

ACC 1:	Acetyl coenzyme A carboxylase
AR:	Adrenergic receptor
CAMP:	Cyclic AMP
DAG:	Diacylglycerol
DNL:	Lipogenesis <i>de novo</i>
FFA:	Free fatty acid
HSL:	Hormone-sensitive lipase
LPL:	Lipoprotein lipase
MAG:	Monoacylglycerol
PDE-3:	Phosphodiesterase-3
PIK:	Phosphatidyl inositol kinase
PKA:	Protein kinase A
PUFA:	Polyunsaturated fatty acids
TAG:	Triacylglycerol
VLDL:	Very low density lipoprotein

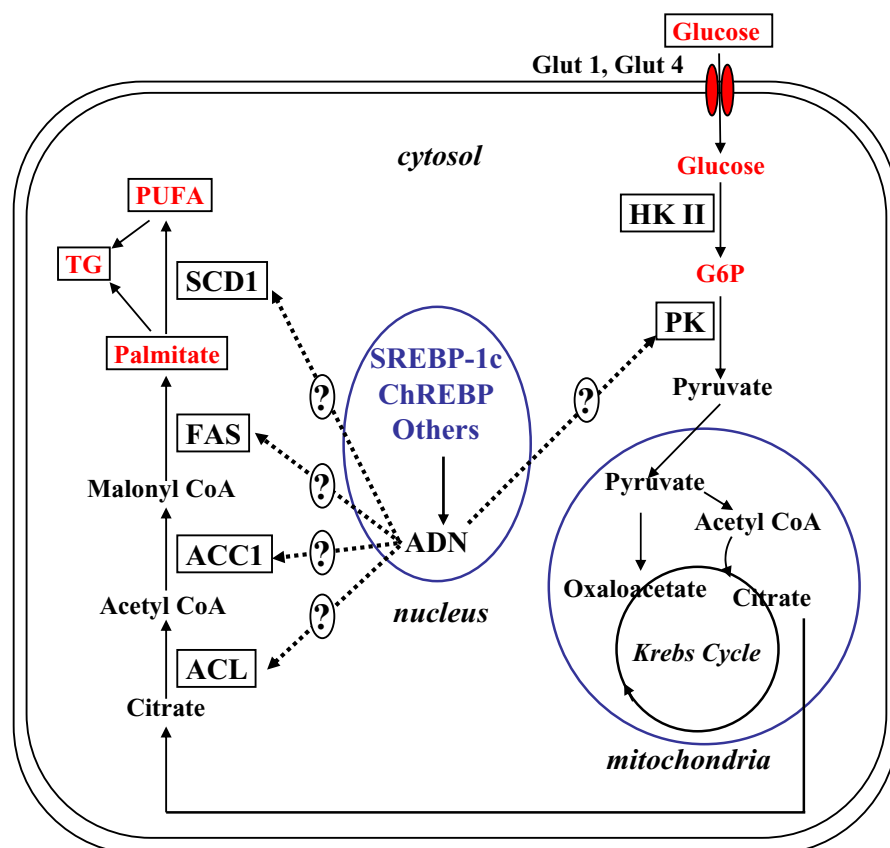


Figure 1

Lipogenic pathway in adipocytes. SREBP-1c is the only transcription factor shown to control the expression of the lipogenic genes in response to insulin in the liver and adipocytes. Recently ChREBP have been proposed as the transcription factor mediating the effect of glucose on the expression of lipogenic genes in rodent liver. Since both SREBP-1c (Sterol Response Element Binding Protein) and ChREBP (Carbohydrate Response Element Binding Protein) are expressed in the adipose tissue, it is possible that they play in this tissue the same role as in the liver. HK II: hexokinase II, PK: pyruvate kinase, ACL: ATP-citrate lyase, ACC1: acetylCoA carboxylase 1, FAS: fatty acid synthase, SCD 1: stearyl CoA desaturase 1, PUFA: polyunsaturated fatty acids, TG: triglycerides.

and in rodent adipocytes in primary culture [25, 26]. The role of SREBP-1c in this response although supported by data obtained in 3T3-L1 adipocytes [27], has been questioned [28] and the transcription factors involved are not yet clearly identified. In human fed with a high carbohydrate diet, neither DNL nor expression of lipogenic genes (FAS, ACC-1 and SREBP-1c) in adipocytes are increased while hepatic DNL is increase 2-3 fold [10, 18]. On the other hand, in human fed with a high fat diet, hepatic DNL is decreased without any changes in the mRNA concentration of the lipogenic genes in adipose tissue [14].

The regulation of DNL in adipocytes and its implication in pathologies such as obesity remain to be fully understood.

Uptake of fatty acids from plasma

Most of the fatty acids used by adipocytes for TAG synthesis are provided by plasma, either as non-esterified fatty acids bound to plasma albumin, or as TAG incorporated in TAG-rich lipoproteins, mainly chylomicrons in the post-prandial state and VLDL in the post-absorptive state. TG-fatty acids must be first released from lipoproteins through the hydrolysis of TAG by the lipoprotein lipase [29]. The expression and activity of LPL in adipose tissue increased in the fed state, particularly during high carbohydrate diet. The uptake of TG-fatty acids depends probably also in part

of the VLDL-receptor, a member of the LDL-receptor family [30], which is expressed in adipose tissue and binds apoprotein E rich lipoprotein such as VLDL, chylomicrons and remnants. Mice deficient in VLDL-R have a reduced fat mass and a resistance to diet-induced obesity and VLDL-R deficiency reduces the obesity of ob/ob mice [31]. VLDL-R binds TG-rich lipoproteins and brings them probably in close contact with LPL [30].

Triacylglycerol synthesis

Fatty acids transport and uptake (Fig 2)

Whatever their origins (plasma albumin bound NEFA in lipoprotein TG), long chain fatty acids can not diffuse passively through plasma membranes and their uptake by adipocytes requires specific processes [32, 33]. The actual mechanism of transmembrane fatty acids flux is still controversial. It is likely that both a passive diffusion through a flip-flop process and a transport by specific protein transporters co-exist in all cells. Human white adipocytes express several fatty acid transporters that facilitate and regulate the transport of fatty acids through the plasma membrane: the protein CD36 (human homologue to murine Fatty Acid Translocase FAT) [34], the Fatty Acid Transport Protein (FATP) [35] and the Fatty Acid Binding Protein, plasma membrane (FABPpm) [36]. The protein FAT/CD36 and

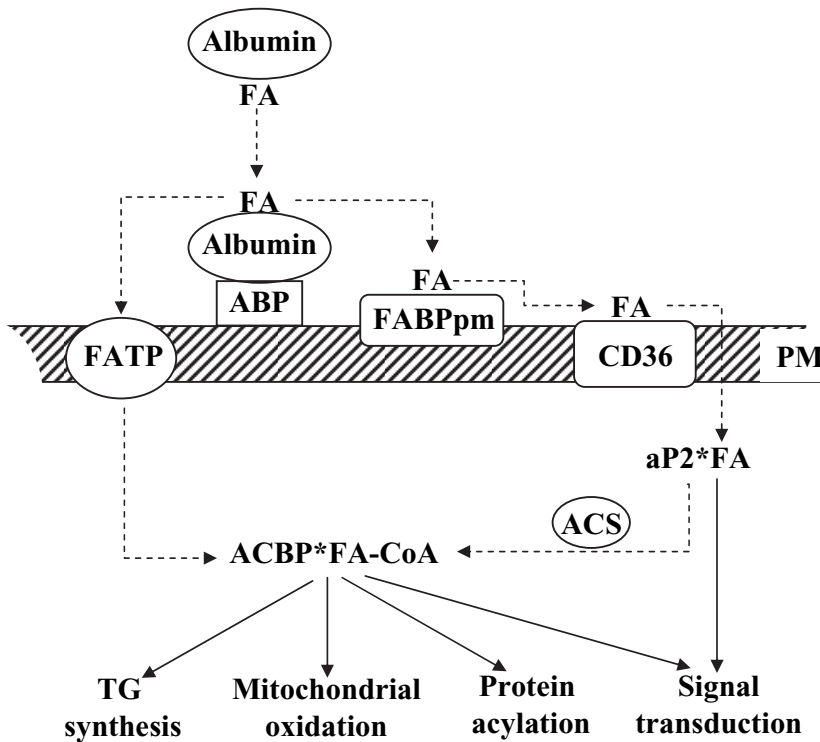


Figure 2

Hypothetical transport of long-chain fatty acids across the plasma membrane and in the cytosol of the adipocytes. FA: long chain fatty acid; PM: plasmalemmal membrane; ABP: albumin-binding protein; FABPpm: plasmalemmal fatty acid binding protein; CD36: human homolog of rodent Fatty Acid Translocase; FATP: fatty acid transport protein; aP2: also called AFABP for adipose fatty acid binding protein; ACS: acylCoA synthetase; FA-CoA: acyl-Coenzyme A; ACBP: acyl-CoA binding protein; TG: triglycerides.

FATP are integral membrane protein while FABPpm is a peripherally membrane bound protein. Recent findings in mouse suggested that the FA transport protein FATP possesses catalytic properties promoting the conversion of FA into fatty acyl CoA and may therefore modulate the uptake of FA by creating an intracellular sink for FA [37]. CD36 is present either in an intracellular pool or in a plasma pool [38]. Studies in rats have shown that in muscle, fatty acids uptake is regulated in rats by translocation of the membrane transporter CD36 from an intracellular pool to the plasma membrane [38] and that this translocation is triggered by contraction and insulin [39]. The latter involves a PI3-kinase mediated mechanism [39,40]. In addition, a permanent relocation of FAT/CD36 to the plasma membrane is associated with an increased FA uptake by adipose tissue of obese Zucker rats, an animal model of insulin-resistance and obesity [41]. This suggests that in animals, malfunction of the FA transport could play a role in the development of disease such as insulin-resistance, diabetes and obesity. In human adipocytes, such intracellular redistribution of the transporter FAT/CD36 and FATP activities may also take place adding an other level of short-term regulation of fatty acid uptake and dysregulation of these mechanisms may also be involved in the development of obesity.

Within the cell, the FA and the lipids in general are carried membrane to membrane or to the acyl CoA synthetase reaction site by cytoplasmic Lipid-Binding Proteins (LBPs) also called Fatty Acid Binding Protein (FABP). Transfer of FA to and from Adipocyte-LBP (ALBP) occurs during protein-membrane interaction only, this mechanism is

identical for human, rat and mouse [42, 43]. Human white adipocytes express two different LBPs: Adipocytes Lipid Binding Protein (ALBP or AFABP or aP2) and Keratinocyte Lipid Binding Protein (KLBP). AP2 is expressed only in white adipocytes while KLBP is expressed in other cell types. In human adipocytes as in rodent, the aP2 protein is more abundant than KLBP (99:1 in normal mouse) and the ratio aP2/KLBP is different in different depots and may have an impact on the metabolic rates of these different fat depots [44].

Production of Acyl-coenzyme and Glycerol-3P (Fig 3)

TAG synthesis requires glycerol and fatty acids which are transformed into glycerol-3P and fatty acyl-CoA to be used as substrates. In healthy human adipocytes and rodent adipocytes, the glycerokinase activity is negligible. The glycerol-3P is therefore produced from glucose through the first step of glycolysis and from glycerogenesis [45]. The glucose enters the adipocytes through the membrane glucose transporters 1 and 4 (respectively Glut1 and Glut4). The cellular glucose uptake is controlled by insulin which promotes the translocation of the intracellular pool of Glut4 to the membrane. Glyceroneogenesis is the production of glycerol-3P from gluconeogenic substrates such as pyruvate. This pathway is an abbreviated version of neoglucogenesis and is mainly regulated at the level of cytosolic PEPCK [46-49]. The proportion of glycerol-3P produced by glycolysis and by glycerogenesis varies with the nutritio-

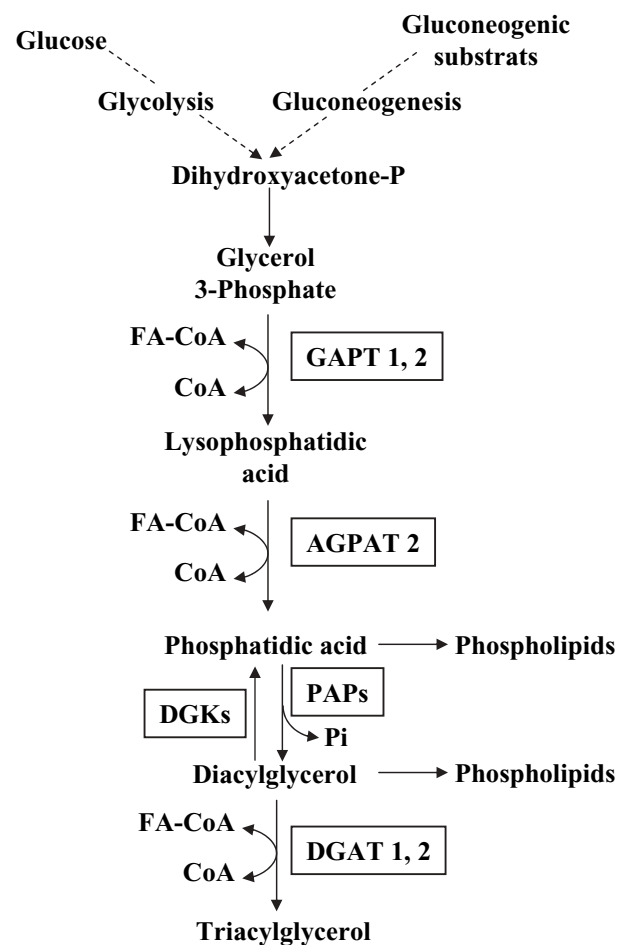


Figure 3 Triglyceride synthesis pathway in adipocytes. FA-CoA: acyl CoenzymeA; GAPT: glycerol-3-phosphate acyltransferase; AGPAT: 1-acylglycerol-3-phosphate acyltransferase; PAPs: phosphatidate phosphohydrolase; DGKs: diacylglycerol kinase; DGAT: diacylglycerol acyltransferase.

nal status (fed state or fasting). Recent studies using genetically modified mice in which the gene for PEPCK-C has been either specifically deleted or overexpressed in adipose tissue have demonstrated the importance of glycerogenesis and PEPCK-C in controlling TAG turnover in mice white adipose tissue [50-52]. Therefore modifications of glucose flux entering the adipocytes and of glyceroneogenesis flux in human adipocytes are likely to have an important impact on TAG turnover in these adipocytes.

In cell cytoplasm, fatty acids and coenzyme are esterified into Acyl-Coenzyme A (acyl-CoA) by acyl-coenzyme A synthetase enzymes. As reported in mouse, the fatty acid transport protein FATP may also function in the human adipocytes as very long chain fatty acyl-CoA Synthase [37]. Medium and long chain acyl-CoA esters are carried within the cells by acyl-CoA binding protein (ACBP) which have a high affinity for these molecules but do not bind FA. Like for FABPs, the exact biochemical functions carried out by ACBP in general and in human adipocytes remain elusive [53, 54].

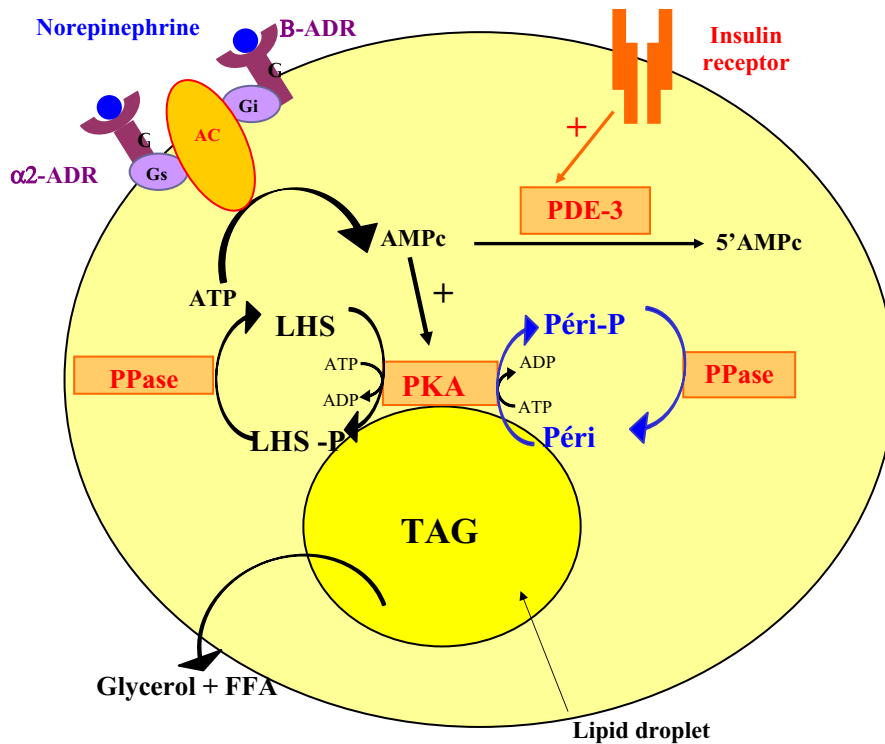
Acyl-coenzymeA esterification of glycerol-3P: TAG synthesis

In adipocytes, the biosynthesis of TAG (*Fig 3*) is the successive esterification of the alcoholic functions of the glycerol-3P by different enzymes respectively named glycerol-3-phosphate acyltransferases (GPATs) [55], 1-acylglycerol-3-phosphate acyltransferase (AGPATs) [56] and diacylglycerol acyltransferases (DGATs) [57, 58]. All the enzymes involved in the synthesis of TAG present different isoforms and are encoded by different genes. In adipose tissues the isoforms GAPT1, GAPT2, AGAPT2, DGAT1 and DGAT2 are present and the most abundant [59] but both the tissue specificity and the substrate specificity of the different isoforms are not completely clear yet. The TAG biosynthesis takes place mainly in microsomes and all the enzymes involved in that pathway are localized there. Although there is evidence in mice for the involvement of the mitochondrial isoform of GPAT in the storage of TAG in adipocytes [60]. The intermediate products of this pathway, lysophosphatidic acid, phosphatidic acid and diacylglycerol (DAG) are involved in important cellular function such as signal transduction [61-64]. Therefore modifications of the activity of these enzymes will have an effect not only on TAG storage but also on other cellular signaling events and biosynthesis of glycerophospholipids.

Lipolysis

During intracellular lipolysis, TAG are broken down in a stepwise fashion via DAG and MAG to form three moles of FFA and one mole of glycerol per mole of completely hydrolyzed TAG. Under most conditions, TAG are completely hydrolyzed although up to 10% can be partly hydrolyzed to MAG and DAG in human fat cells. This incomplete lipolysis may lead to accumulation of the partial acylglycerol or lipolysis can start from DAG [62].

The rate-limiting enzyme for hydrolysis of adipose tissue TAG to DAG and MAG is the **hormone-sensitive lipase** (HSL; E.C. 3.1.13), so named because of its responsiveness to hormones: insulin and catecholamines [66]. HSL is subject to intense regulation, acutely via reversible phosphorylation. The different steps of the lipolytic process leading to the activation of HSL (the so-called lipolytic cascade) are well defined (*Fig 4*). The first cellular action of catecholamines and of a number of endocrine and/or paracrine regulators of lipolysis (e.g. adenosine and prostaglandins) is their binding to plasma membrane receptors. As reviewed previously [67], catecholamines are maybe the most sophisticated regulators of fat cells function, since they regulate lipolysis from separate adrenergic receptors (AR). Three subtypes of η -ARs (η 1,2,3) are positively coupled to adenylyl cyclase by the stimulatory G-protein complex (Gs) and stimulate lipolysis. The ζ 2-AR is negatively coupled to

**Figure 4**

The lipolytic cascade in adipocyte. ADR: adrenoceptor, AC: adenylyl cyclase, FFA: free fatty acid, G: G protein, LHS: hormone-sensitive lipase, PDE: phosphodiesterase, Peri: perilipin, PKA: protein kinase A, PPase: protein phosphatase, TAG: triacylglycerol.

the enzyme by the inhibitory G-protein (Gi) and inhibits lipolysis. The stimulation of adenylyl cyclase leads to an increment of intracellular cyclic AMP (cAMP) concentrations which in turns, promotes the activation of cAMP-dependent protein kinase A (PKA) which activates HSL and results in the hydrolyze of TAG into DAG and MAG.

The MAG are finally hydrolyzed into FFA and glycerol by a nonspecific monoacylglycerol lipase which exists in abundance and is not regulated by hormones [68]. The adipocyte cAMP level and thereby lipolysis can also be regulated by the enzyme phosphodiesterase III (PDE-3), which breaks down cAMP to 5'AMP [69]. The lipolysis end-product glycerol is poorly re-utilized by adipose tissue and is transported in the circulation to others tissues (principally liver) in order to be metabolized. The release of glycerol from adipocyte is dependent in part of a channel-forming integral protein of the cell membrane, aquaporin adipose (AQPap). AQPap is a member of a family of at least 11 proteins that functions as water channel [70]. Among these, AQP3, present in kidney, AQP9, expressed in liver, and AQPap can transport also glycerol. AQPap expression is enhanced by fasting and repressed by refeeding and by insulin [71]. Missense mutations resulting in the loss of activity to transport glycerol and water have been described [72]. One subject homozygous for one of these mutations had a normal body weight, normal plasma glycerol concentration in the basal state but a lack of increase during exercise. This suggests that AQPap plays indeed a role in glycerol efflux from adipocytes but that another mechanism exists also.

The mobilization of the other lipolysis end-product FFA involves first a passage through the fat cell membrane. The nature of this process is still debated. Lateral diffusion of FFA or utilization of specific fatty acid binding proteins (FABP) in adipocytes (e.g. ALBP also known as aP2 or KLBP) [73, 74] are common theories. After leaving the fat cell membrane, the FFA bind to carrier proteins in order to be transported in the interstitial space and in the circulation since FFA are water-insoluble. Several FFA-carrier protein are discovered but the most important one is albumin [75].

Although it is generally accepted that lipolysis is stimulated by PKA activation which phosphorylates HSL, there is accumulating evidence to suggest that, in addition to PKA, G protein-coupled receptors and cyclic AMP can also activate the mitogen-activated protein kinase (MAPK) pathways and extracellular signal-regulated kinase (ERK) [76]. The phosphorylation and activation of HSL by ERK provides an additional pathway and mechanism for the regulation of HSL and the control of lipolysis. Additional studies will be required to investigate this new pathway.

Recent data suggest that other proteins are important in regulated lipolysis, such as the most abundant phosphoprotein of adipocyte lipid droplets: the perilipins. The role of these proteins (HSL and perilipins) which are both phosphorylated by PKA will be discussed further below.

Hormone-sensitive lipase

HSL was primarily discovered and characterized in rat adipose tissue as a 84 kDa protein corresponding to a

768 amino acid protein [77]. In human adipose tissue, HSL was detected as a 88 immunoreactive kDa protein with 775 aminoacids encoded by nine exons spanning eleven kilobases and its gene mapped to chromosome 19 cent-q13.3 [78]. However a truncated catalytically inactive form of HSL generated through in-frame skipping of exon 6 during the processing of HSL mRNA, has been described in human but not in rat adipose tissue [79]. Recently, we showed in some obese subjects the presence of this shorter variant of HSL (80 instead of 88 Kda). The presence of this variant in abdominal subcutaneous adipocytes is associated with an impaired adipocyte lipolysis: decreased of HSL activity and *in vitro* maximally lipolytic capacities of isolated adipocytes [80].

HSL is present in other tissues than white adipose tissues like adrenals, ovaries, testes, brown adipose tissue, pancreatic islets, heart and skeletal muscles. In addition to its major role in FFA mobilization, it plays in steroidogenic tissues an important role of cholesteryl ester hydrolase in steroid production. Evidence is provided to demonstrate that rat HSL exists as a functional dimer composed of homologous subunits. Dimeric HSL displayed approximately 40-fold greater activity against cholesteryl ester substrate when compared with monomeric HSL without any differences in affinity for the substrate [81].

HSL is the only TAG lipase and cholesteryl ester hydrolase in which the activity is controlled through phosphorylation (the hydrolytic activity against DAG and MAG is unaffected by phosphorylation). The PKA activation leads to the phosphorylation of a serine residue called the « regulatory site » (Ser-563) [82]. A further « basal site » Ser-565 of HSL is phosphorylated *in vivo* [83] but it is not yet clear under which condition, if any its extent of phosphorylation changes. Subsequently two further PKA phosphorylation sites Ser-659 and Ser-660 have been discovered [84]. The catalytic triad of HSL has been identified by site directed mutagenesis and consisted in three aminoacids: Ser-423, Asp-703 and His 733 and Ser-423 (numbered for rat HSL). Phosphorylation of HSL *in vitro* with exogenous PKA, causes a modest 2-fold activation of HSL whereas in intact cells a 30- to 100-fold increases specific activity of HSL is observed upon elevation of PKA activity. To explain this discrepancy, Brasaemle *et al.* proposed that PKA-translocation of HSL from the cytosol to the surface of lipid droplet explains the large cellular response to lipolytic stimuli [85]. Recently, Su *et al.* demonstrated that Ser-659 and Ser-660 within HSL is required to effect the translocation reaction as well as phosphorylation of the catalytic Ser-423 [86].

The first description of HSL-deficient mice has been reported in 2000 [87]. The main feature of the phenotype is male sterility because of oligospermia demonstrating that the testicular form of HSL is required for spermatogenesis. White adipocytes from these animals are very large but the

mice are not obese. In white adipose tissue, *in vitro* TAG lipase activity and catecholamine-induced lipolysis are decreased but not entirely abolished. Furthermore, the HSL \neg show DAG accumulation in adipocytes, muscle and testis. These findings suggest that HSL is the rate-limiting enzyme for DAG hydrolysis in adipose tissue. The residual lipolytic activity might be explained by the presence of one or more additional intracellular TAG lipase(s) at least in the knock-out mice. Further investigation need to be performed in order to determine the molecular species of this (ese) new lipase(s) [88].

The perilipins

Perilipins are a family of phosphoproteins that coat the surfaces of intracellular storage droplets in brown and white adipocytes and steroidogenic cells where lipolysis appears to be uniquely sensitive to regulation that is mediated by PKA [89]. Perilipins are not found in other types of cells that contain neutral lipid droplets such as hepatocytes, Ito cells and alveolar cells in mammary glands from lactating rats [90]. The abundance of perilipin is approximately 0.25-0.5% of total cellular protein. They were first identified as an approximately 60 kDa phosphoprotein of rat adipocyte that is phosphorylated by PKA upon stimulation of lipolysis [91].

Perilipins are encoded by a single gene (*Plin*) and the protein expression is increased during the differentiation of preadipocytes to adipocytes. Alternative mRNA splicing results in two protein isoforms in rat [92]. Perilipin A (58 kDa) and perilipin B (46 kDa) share a common N terminal domain of 406 amino acids and distinct carboxy terminal domains: a sequence of 111 amino acids for perilipin A and only 16 amino acids for perilipin B. They have 40% sequence identity through their first 120 amino acids with ADRP (adipose differentiated-related protein), another lipid droplet-associated protein [92].

Subsequent analyses confirmed the presence of perilipins in other mammalian species: mouse [92], human [93, 94] and bovine. In mice, four perilipin species are encoded by a single copy gene generated by alternative pre-RNA splicing and polyadenylation [95]. The perilipins A and B are expressed in adipocytes and steroidogenic cells but perilipins C and D expression is limited to the latter population. The four perilipin proteins have a common N-terminus but unique C-termini.

The perilipins bind tightly to lipid droplets. The use of detergent is required to remove perilipins from lipids droplets as this is probably due to the presence in perilipins A and B of 3 regions of 20 aminoacids with moderate hydrophobic characters and 5 domains of 10 to 11 aminoacid sequences with characteristics of amphipathic η -pleated sheets. Cytochemical analysis by immunogold labeling suggest that the perilipins are located on or within the limiting surface of the droplet [90].

Functions of perilipins

When expressed in fibroblastic 3T3-L1 preadipocytes, periA targets to intracellular neutral droplets, where it protects TAG against hydrolysis and increases storage of TAG [96].

Target disruption of Plin results in healthy mice that have constitutively activated fat cell HSL. With a higher food consumption in peri null mice, no differences in body weight among the genotypes were reported but the peri null mice contained less adipose tissue and more muscles than wild type mice. They are also resistant to diet-induced obesity [97]. A more recent study showed similar results except that identical food consumption was reported among the genotypes and that peri null mice showed an increased tendency to develop glucose intolerance and peripheral insulin resistance [98].

The perilipins are multiply phosphorylated in response to increase intracellular cAMP. The common region contains three consensus sites for phosphorylation by PKA, and three additional PKA sites are located within the unique region of the A, but not the B isoform [99]. The coordinate phosphorylation of HSL and the perilipins accompanies the induction of lipolysis thus suggesting a role for perilipins in the process. Phosphorylated perilipin may serve as a docking protein for hormone-sensitive

lipase, allowing association of the lipase only when the cells are hormonally stimulated. Alternatively, conformational changes of phosphorylated perilipins may expose the neutral lipid core of the lipid droplets, facilitating the ensuing hydrolysis (Fig 5).

Little is known about the regulation of perilipin expression. Souza *et al.* showed that tumor necrosis factor- ζ (TNF) downregulates periA mRNA and protein expression in 3T3-L1 in association with a rise in lipolysis [100]. More recently, it was reported that the perilipin gene expression in differentiating adipocytes is regulated by PPAR γ 2 [101]. Finally, overexpression of leptin in transgenic mice leads to decreased basal lipolysis and perilipin levels [102].

Regulation of lipolysis

Regulation of lipolysis is essential to ensure an adequate supply of lipid to the tissues that utilized FFA. Several hormonal para-endocrine and extra hormonal factors participate in the regulation of human lipolysis.

Stimulation of lipolysis

Contrary to most species, catecholamines are the only hormones to markedly stimulate lipolysis in man. These hormones may reach adipose tissue via the general circula-

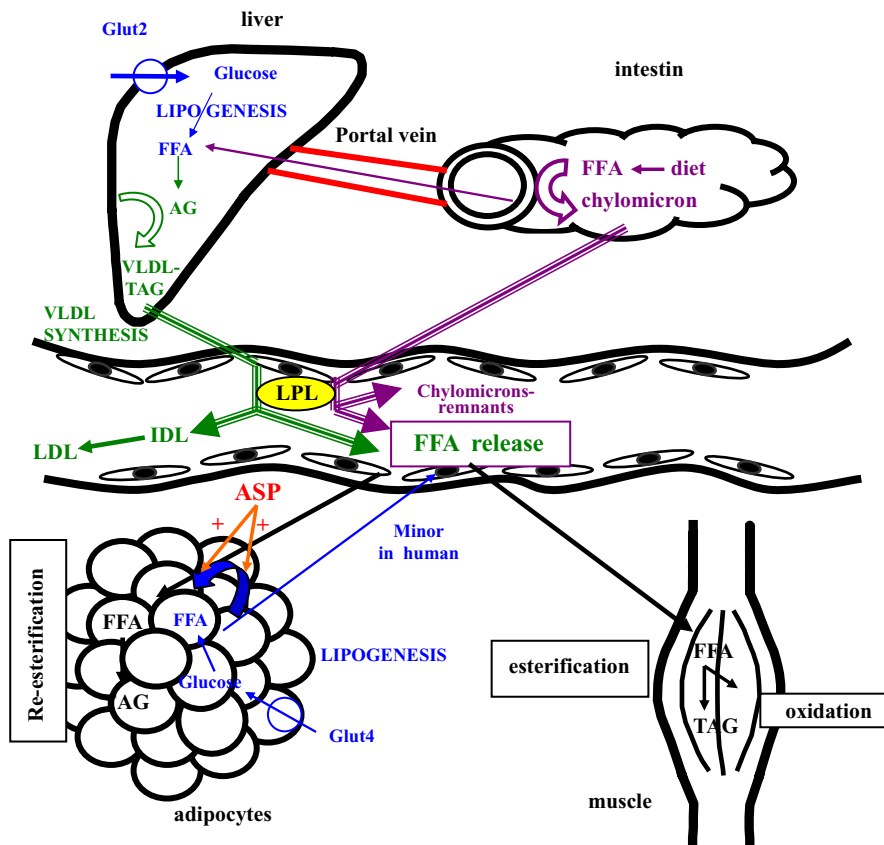


Figure 5

Schematic illustration of fatty acid release after a meal. See "Physiological modulation of TAG metabolism in post-prandial, post-absorptive, and starvation states" in the text for more details. ASP: acylation-stimulated protein, CPT 1: carnitine palmitoyl transferase 1, FFA, Free fatty acid; Glut 2: glucose transporter II, Glut 4: glucose transporter IV, IDL: intermediate-density lipoprotein, LDL: low-density lipoprotein, LPL, lipoprotein lipase, TAG, triacylglycerol, VLDL: very-low-density lipoprotein.

tion (mainly adrenaline) [103] or via the sympathetic innervation (noradrenaline) [104]. Their action is mediated by the three different η -AR subtypes which activate the lipolytic cascade (as discussed above). *In vivo* data using microdialysis technique, show that the three AR subtypes are involved in the *in vivo* regulation of human lipolysis [105]. In contrast to other species, *in vivo* experiments have suggested that catecholamines-induced lipolysis is predominantly mediated by η 2-ARs, at least in healthy subjects [106]. While the involvement of η 3-AR subtype in lipolysis is well documented in laboratory animals [67], the relative importance of this η -AR subtype in man is less clear [105]. There is evidence that η 3-ARs is mainly expressed in visceral fat, where it may play a pathogenic role in upper-body obesity [103]. However, this receptor seems to be of minor importance for stimulating lipolysis in subcutaneous fat.

Other hormones may stimulate lipolysis through Gs coupled receptors in a similar way as catecholamines. However, the effect is small and their physiological and pathophysiological role for lipolysis regulation is unclear so far. These hormones includes thyroid stimulating hormone (TSH) [107], glucagon [108], cholecystokinin [107].

A number of hormones have so-called permissive effects on catecholamine-induced lipolysis in human fat cells, such as glucocorticoids and thyroid hormones [111, 112] as well as growth hormone [113, 114] and sex steroid hormones [115]. It is speculated that circulating testosterone may influence lipolysis in abdominal obesity [116]. However, a normalization of free testosterone in polycystic ovary syndrome has no influence on lipolysis regulation [117].

In addition, the cytokine tumor necrosis factor alpha (TNF) is expressed in human adipocytes, and may play a role in regulation of lipolysis probably as a paracrine factor. This cytokine has been shown to increase the lipolysis rate in humans *in vivo* [118] and in primary cultures of newly differentiated human preadipocytes [119]. The TNF production is increased in obesity and does respond to insulin and other energy-balance signals [120].

The role of glucose on the regulation of lipolysis is still unclear. The exposure of T3-F442A adipocytes for 32h to a medium without glucose led to a decrease in HSL mRNA and HSL total activity, which was reversed by re-administration of glucose for 12 h [121]. Prolonged treatment of adipocytes with high glucose (20 mM) and insulin concentrations (25 mg/ml) increases basal and stimulated lipolysis associated with a 40% increase in the level of HSL [122]. However, neither insulin nor glucose alone affected basal and maximally stimulated lipolysis. The molecular mechanisms underlying the effect of glucose on HSL gene expression could be the presence of a glucose-responsive region within the HSL promoter [123].

Inhibition of lipolysis

Insulin is the most potent antilipolytic hormone in adipose tissue, as in other tissues. Insulin is central for the

regulation of the anabolic actions of fat cells, by stimulating glucose uptake, stimulating FFA uptake via action of LPL on circulating TAG, inhibiting lipolysis, and possibly by stimulating *de novo* fatty acid synthesis [as reviewed, 124]. Lipolysis in normal subjects is extremely sensitive to insulin action. Local adipocyte lipolysis decreases within 15 minutes after the start of an insulin infusion [124]. When insulin is infused to moderately high physiological concentrations, there is therefore complete suppression of nonesterified fatty acid release from adipose tissue. The details of the insulin-receptor signaling system, including the mechanisms underlying inhibition of lipolysis are not fully understood. The first step in insulin action is binding to specific cell-surface receptors. Thereafter, a number of intracellular processes are initiated through receptor autophosphorylation by tyrosine kinase activation and further phosphorylation of several intracellular insulin receptor substrates [125]. The insulin action on specific steps in the lipolytic cascade (Fig 4) is exerted through a stimulation of the phosphodiesterase subtype PDE-3, causing a lowering in the intracellular level of the intracellular level of cAMP [126]. *In vivo*, selective inhibition of PDE-3 can completely abolish the antilipolytic effect in human adipose tissue [127]. PDE-3 may be stimulated by an insulin receptor signaling through several intracellular phosphorylation steps, leading to an activation of a phosphatidylinositol kinase 3 (PIK-3) [128]. Dephosphorylation of HSL may further be enhanced through insulin mediated activation of a protein phosphatase [129]. Additional mechanisms may exist. Insulin can inhibit adenylyl cyclase [130] and insulin may internalize η -ARs in human fat cells, a mechanism that may contribute to insulin-induced lipolytic catecholamine resistance [131].

The net effect of catecholamines on lipolysis depends on the functional balance between the stimulating η -ARs and the inhibiting ζ 2-AR. In most of *in vivo* and *in vitro* studies, stimulation of η -AR in adipose tissue dominates over ζ 2-AR inhibition of lipolysis. The physiological relevance of ζ 2-AR for lipolysis is less clear than for η 1 and η 2-ARs. Results of *in vivo* studies using *in situ* microdialysis methods suggest that ζ 2-ARs could be involved in the modulation of lipolysis at rest or when plasma epinephrine levels are increased (e.g. mental stress) [132]. The number of ζ 2-ARs seems to be directly correlated with cell size and a reduction in adipocyte size (e.g. promoted by fasting) has been associated with a reduction in ζ 2-ARs responsiveness [133].

Inhibition of lipolysis is also induced *in vivo* by insulin-like growth factor-1 (IGF 1) in human [134] but its physiological relevance is still uncertain. IGF 1 probably inhibit lipolysis through the PIK-3/PDE-3 pathway in a similar fashion as insulin.

Adenosine is produced by adipose tissue and has been shown to inhibit HSL even if adenosine concentrations are low *in vivo* [135]. The high production rate *in vitro* relative to *in vivo* experiments has been suggested to be the consequence of damaged cell secretion [136]. *In vitro* experiments

have shown that adenosine has its own receptors (A receptors) [137] that inhibit adenylyl cyclase activity *via* Gi proteins with a mechanism identical to the ζ 2-AR described above [138]. It has been suggested that adenosine inhibits lipolysis both as a paracrine and autocrine agent, regulating lipolysis locally.

Prostaglandins E₁ and E₂ have also been shown to be potent inhibitors of lipolysis in human adipose tissue *in vitro*, following binding to cell surface membrane [139]. Like for adenosine, the effect is mediated by Gi proteins which inhibit adenylyl cyclase [140]. The lipolysis inhibition has been difficult to assess *in vivo* because the prostaglandin synthesis by adipocytes is very low [140].

Neuropeptide Y and peptide YY have been shown to inhibit adenylyl cyclase and lipolysis in human adipocytes. Their action is mediated by Gi proteins [141]. Although the significance of the action of peptide YY is still unclear since it is a gut peptide, it appears more obvious for neuropeptide Y which is synthesized by the same sympathetic nerve terminals as norepinephrine [142]. Comparison of the maximum antilipolytic effect of neuropeptide Y, adenosine analogues and prostaglandins E₁, showed that the lipolysis inhibition response with neuropeptide Y is half of the response with the other agents [143].

Finally, the ketone bodies acetoacetate and η -hydroxybutyrate may inhibit lipolysis, decreasing FFA flux by about 75% of the basal rate in postabsorptive subjects [144]. The mechanism and role of ketone bodies in lipolysis are still uncertain [145].

Basal lipolysis

When human fat cells are incubated *in vitro* in the absence of any stimulatory agents, a spontaneous lipolytic activity called basal lipolysis is measured, ranged from 0.3-1.0 σ mol of glycerol/h/g fat tissue [146]. Unlike human cells, basal lipolysis is usually undetectable in animal adipose tissue of most laboratory animals. An artefact of the *in vitro* incubation system seems unlikely since a basal lipolytic activity was also found *in vivo*. As a matter of fact, lipolytic capacity is not completely abolished by a simultaneously local perfusion of selective η - and ζ -AR antagonists [147]. The mechanisms responsible for the maintenance of the basal lipolysis rate are still unknown. However a number of studies showed that fat cell size could be of importance for the rate of basal lipolysis, since a positive correlation was found between the basal rate of lipolysis and the fat cell size [146].

Regional differences in lipolysis

Many studies have shown that human adipose tissue is heterogeneous with respect to responsiveness and metabolism. Site differences in lipolysis have been demonstrated *in vitro* and *in vivo* (microdialysis of peripheral- and abdominal subcutaneous fat depots, turnover experiments) in nor-

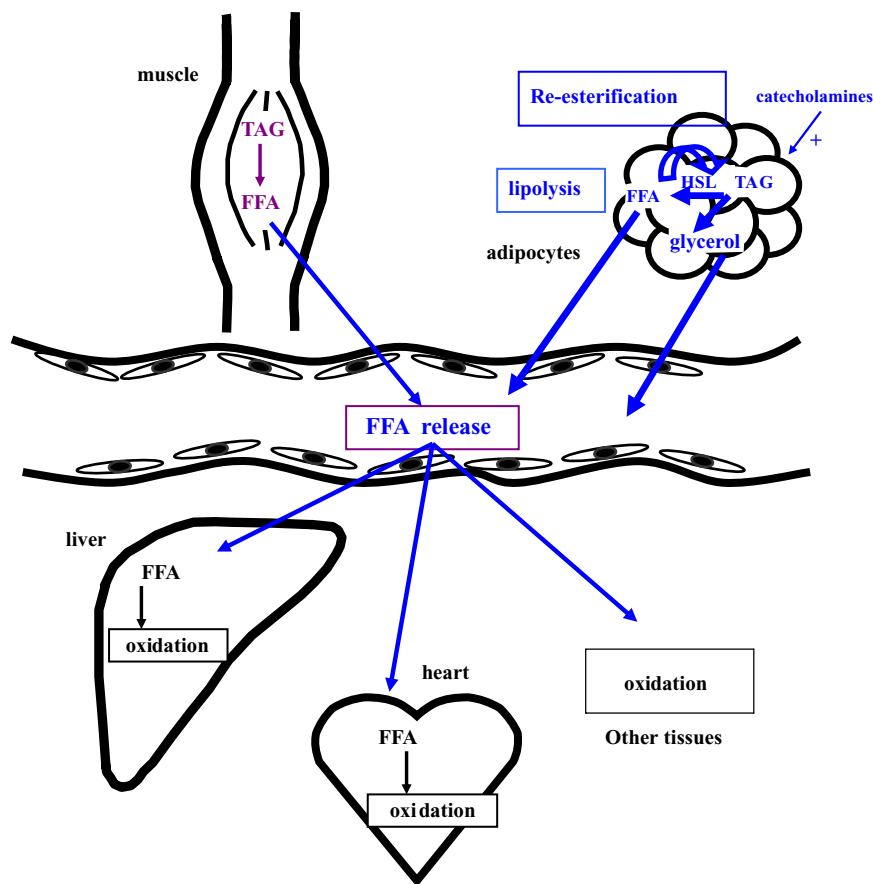
mal-weight subjects. In both men and women, the highest lipolytic activity of catecholamines was found in the visceral fat depot, followed by the abdominal subcutaneous region (the major body fat depot) and the lowest activity in the peripheral subcutaneous fat depots (i.e. gluteal and femoral) [148]. The antilipolytic effect of insulin in omental tissue is lower than in subcutaneous tissue [149]. Moreover, adenosine and prostaglandins E seem to contribute to a decreased antilipolytic function of the omental adipocytes as compared to the abdominal subcutaneous adipocytes [138].

In vitro experiments suggested that the mechanisms behind the discrepancy between visceral and subcutaneous fat resides above all, at the level of hormone receptor function. Increased catecholamine-induced lipolysis in visceral fat cells seems to be mainly due to increased expression of η 1- and η 2-ARs [150, 151] and enhancement of the η 3-AR function [152]. Moreover, decrease of antilipolytic action of insulin appears to be linked to a decrease insulin receptor affinity, combined to an impaired post-receptor inhibition of the lipolysis rate [149]. Adenosine and prostaglandins E have a decreased antilipolytic action in visceral fat depot which probably linked to a decreased receptor expression in these adipocytes as compared to abdominal subcutaneous adipocytes [3, 138, 146].

The regional differences in subcutaneous fat depots are more pronounced in women than in men, which may contribute to the well-known gender differences in accumulation of fat [3, 148]. The regional differences in lipolysis between the different fat depots are also more pronounced in obese subjects and are influenced by physiological modulation of lipolysis, as further discussed below.

Physiological modulation of tag metabolism in post-prandial, post-absorptive, and starvation states

After a meal (*Fig 6*), dietary fat is absorbed by the intestine and secreted into the circulation in the form of chylomicrons. Some FA are also synthesized in the liver and the adipocytes through *de novo* lipogenesis. In the adipocytes, most of the newly synthesized FA are stored as TAG. In the liver, the newly synthesized FA are preferentially directed into TAG synthesis rather than mitochondrial oxidation. The carnitine palmitoyl transferase I (CPT 1) which catalyses the formation of long-chain acyl-carnitine from activated fatty acids and carnitine, thus committing them to oxidation, is inhibited by high tissue malonyl-CoA content [153]. The newly synthesized FA are secreted as TAG incorporated into very low density lipoproteins (VLDL). When reaching the capillary endothelium, the TAG in the chylomicrons and the VLDL are massively hydrolyzed by the lipoprotein lipase (LPL) releasing the FA which are taken up by the muscle (mainly for oxidation) and above all, the adipocytes (mainly

**Figure 6**

Schematic illustration of FFA release in the post-absorptive state. See "Physiological modulation of TAG metabolism in post-prandial, post-absorptive, and starvation states" in the text for more details. FFA, Free fatty acid; HSL: hormone-sensitive lipase, TAG, triacylglycerol.

for storage through TAG synthesis). Whilst LPL is activated, HSL activity is rapidly suppressed by dephosphorylation after a meal [154]. This is probably linked to the rapid increase of circulating insulin concentration in combination with an increase in adrenoceptor affinity *in situ* [155, 156]. Fat deposition in adipose tissue seems to be stimulated by a 76 amino acid fragment called acylation stimulating protein (ASP), produced by both murine and human adipocyte. ASP increases the rate of TAG synthesis [157] by i) stimulating diacylglycerol acyltransferase, the enzyme that regulates the last step in TAG synthesis [158] and by ii) increasing specific membrane transport of glucose via translocation of intracellular glucose transporters to the plasma membrane surface [159]. Saleh *et al.* [157] showed that ASP is generated *in vivo* by human adipocytes and that this process is accentuated postprandially, supporting the concept that ASP plays an important role in clearance of TAG from plasma and fatty acid storage in adipose tissue. Some FFAs never leave the fat cells and are re-esterified to new adipocyte TAG. The estimation of this intra-cellular pool of re-esterification is difficult to determine. The re-esterification process is a form of futile cycle which could be of importance when there is a net storage of energy (i.e. after food intake) [160].

Between meals, in post-absorptive (overnight fasted) and fasting situations, there is a high demand of FFA, and

lipid oxidation can account for more than 70% of total body energy expenditure. Then FFA is the primary oxidative fuel for liver, renal cortex, resting skeletal muscle and heart [161]. The fasting state is accompanied by increased lipolysis activity, which in turn is caused by a number of factors: a nocturnal increase of lipolysis rate, an increase of basal lipolysis rate (*in vitro* experiments only), a decrease of circulating insulin concentrations, and an increased lipolytic action of catecholamines; the latter is in part attributed to the increase of η -AR number and the decrease of β -2-AR number in fat cells. Leaving the adipocytes, the FA circulate in plasma as FFA bound to plasma albumin to be utilized principally by adipocytes (re-esterification into TAG), muscle (oxidation) and liver (oxidation and also TAG synthesis and VLDL secretion).

Pathological modulation of tag metabolism in obesity

Obesity, generally defined as a body mass index (BMI) of ≥ 30 kg/m² is a major health problem in industrialized countries since it is a risk factor for insulin resistance, hypertension, diabetes and cardiovascular diseases. Despite recent discoveries concerning the regulation of energy

metabolism, little is known about the pathogenesis of obesity. Actually, obesity can be viewed as an energy storage disorder with an excess amount of total adipose tissue TAG. However, it is well established that circulating FFA level is increased [146]. This apparent discrepancy between FFA and TAG accumulation is unclear. However there are problems of determination of the true lipolysis rate in obesity in relation to regional lipolysis differences, sex differences, upper or lower obesity, and the determination of the true denominator to express lipolysis (per cell number, per unit lipid weight, per cell surface area). *In vivo*, after an overnight fast, lipolysis rate related to lean body mass is increased in obesity but a decrease is reported when it is related to fat mass. *In situ*, with microdialysis, no modification of the lipolysis rate was found in relation to obesity, after an overnight fast in subcutaneous adipose tissue [162]. *In vitro*, obesity is accompanied by an increased basal lipolysis in abdominal subcutaneous adipocytes, when expressed per number of cells, but nearly normal values were found when related to the weight of fat cells. One possible explanation is the strong influence of adipocyte size on basal lipolysis rate, that increase with the adipocyte size [163]. The hormonal regulation of lipolysis in obesity is under debate. As regards insulin, increased, normal or even decreased antilipolysis effects are reported [163]. This discrepancy could be due to the difficulties in establishing an optimal experimental condition for determining the true antilipolytic effect of insulin as discussed [163]. On the other hand, there is a strong evidence both *in vivo* and *in vitro*, for the existence of lipolytic resistance to catecholamines, in particular in upper-body obese subjects [163]. It has been demonstrated that even children with obesity are resistant to the action of catecholamines on lipolysis *in vivo* [164]. Concerning *in vitro* studies, a lipolytic catecholamine resistance was reported in abdominal subcutaneous fat cells of women and men with upper-body obesity, in relation with a decreased cell surface density of η 2-ARs [165]. However, in visceral fat cells of upper-body obesity, catecholamine-induced lipolysis is augmented and it is often considered that increased mobilization of FFA from the visceral fat depot to the liver is of pathophysiological importance for the development of the metabolic syndrome [163]. This is linked to an increased η 3-AR function and a decreased ζ 2-AR function in visceral fat cells of obese subjects. Both observations are more marked in men than in women, leading to sex differences in visceral fat lipolysis, which could explain the higher risk of metabolic disorders in obese men than in obese women [166]. Regarding balances between hormones, insulin and cortisol levels are often increased and testosterone and GH levels decreased, leading to an accumulation of visceral fat [167].

Regarding molecular mechanisms behind regulation of lipolysis in adipocytes from severe obese subjects, we have demonstrated that a deficit in subcutaneous adipocyte expression and function of HSL is present in obesity. This

defect is accompanied by a decreased lipolytic capacity of adipocytes when lipolysis is expressed per gram of fat cells [168]. Moreover, because of the increased TNF levels in adipose tissue of obese humans, and since perilipin expression is downregulated by TNF, Wang *et al.* could speculated that elevated production of TNF in obesity may increase basal lipolysis through its effects on perilipin expression or activity [169].

As reviewed before, LPL activity is a key regulator of fat accumulation in various adipose areas, since human adipose tissues derives most of its lipids for storage from circulating TAG (from chylomicra and VLDL). Studies in humans have revealed increased adipose tissue LPL activity with obesity which could increase NEFA re-esterification to TAG in adipocytes and increase the storage of lipids in adipocytes of obese subjects [168].

Conclusions

Research carried out in the past few years has clearly increased our understanding of the metabolism of lipids and its regulation in human (obese or non-obese). However, lipid regulation is still extremely complex due to the large number of organs implicated in this process, the heterogeneity of fat depots (mainly visceral and abdominal subcutaneous), sex differences and the diversity of the hormones and other factors that govern this process. Novel identified proteins such as perilipins, ASP suggested an important role but a number of questions have also risen from these studies that need to be investigated in more details.

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