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FATTY ACID FLUX IN ADIPOCYTES; THE IN'S AND OUT'S OF FAT CELL LIPID TRAFFICKING

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Abstract

The trafficking of fatty acids into and out of adipocytes is regulated by a complex series of proteins and enzymes and is under control by a variety of hormonal and metabolic factors. The biochemical basis of fatty acid influx, despite its widespread appreciation, remains enigmatic with regard to the biophysical and biochemical properties that facilitate long chain fatty acid uptake. Fatty acid efflux is initiated by hormonally controlled lipolysis of the droplet stores and produces fatty acids that must transit from their site of production to the plasma membrane and subsequently out of the cells. This review will focus on the “in’s and out’s” of fatty acid trafficking and summarize the current concepts in the field.

Keywords

Adipocytes; fatty acid; trafficking; influx; efflux

INTRODUCTION

The adipocyte has evolved as a specialized cell type for the storage and release of fatty acids. Adipocytes are unique in that they can accommodate without deleterious effects the massive storage of triacylglycerol (TAG) during energy abundance and releases free fatty acids into the plasma for the use by other tissues during times of energy need. This process of fatty acid uptake and storage balanced by lipolysis is a highly regulated process that takes cues from nutritional and efferent signals to store and supply energy as the body dictates. The adipocyte has a unique cellular organization as well, with greater than 90% of the cell volume being TAG. This results in limited cytosolic space and a contiguous ER, nuclear, plasma membrane interface. This geometry may accommodate the transport of hydrophobic molecules, such as fatty acids and fatty acyl-CoA's to and from the membrane during uptake and lipolysis. The insolubility of fatty acids may also be accommodated by intracellular carriers such as the fatty acid binding proteins and acyl-CoA binding proteins. The exact location and mechanism of trafficking these hydrophobic molecules is under debate but is an important factor when discussing adipocyte storage and lipolysis of TAG.

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FATTY ACID UPTAKE

Long-chain fatty acids (LCFA) transported across the plasma membrane of adipocytes are derived from circulating plasma LCFA's generated by lipoprotein lipase catalyzed hydrolysis of triglycerides in chylomicra or in very low-density lipoproteins [1]. Although most circulating LCFAs are bound to serum albumin, there is a relatively small fraction of unbound long-chain fatty acid (LCFA_u) that is the moiety transported across membranes. The mechanism by which LCFA_u are transported across membranes has been an area of interest as well as controversy. The controversy stems from a debate as to whether cellular LCFA uptake is a diffusion-mediated or a protein-mediated process.

Influx of LCFA involves five steps: 1) dissociation from serum albumin, 2) diffusion through the outer aqueous phase, 3) insertion into the outer leaflet of the membrane, 4) translocation or flip-flop from the outer to the inner leaflet, and 5) dissociation from the inner leaflet into the inner aqueous phase [2,3]. The LCFA_u concentration is proportional to the rate of dissociation of LCFA from serum albumin that in turn is a factor of LCFA structure and chain-length [4]. In dissociation-mediated LCFA uptake, influx rates increase with increasing concentrations of LCFA_u. The most controversial step in membrane transport of LCFA is Step 4 or fatty acid translocation and the question of it being diffusion-mediated or protein-mediated. The transport of a hydrophobic LCFA is argued to involve an extremely rapid (<5ms) passive transport across the lipid bilayer (flip-flop) followed by a slower rate-limiting dissociation of the lipid from the membrane into the intracellular aqueous phase. This concept is based on studies that demonstrate rapid fatty acid flip-flop rates in small unilamellar vesicles [5]. However, cellular membranes have complex lipid and protein structures on their surfaces as compared to smaller membrane vesicles [6] and together with lower substrate availability due to non-saturating physiological LCFA_u concentrations could argue that lipid uptake is predominately protein-mediated. Fatty acid transporters could bind LCFA_u and facilitate influx thereby overriding slower rates of fatty acid flip-flop and dissociation from the membrane bilayer [7]. One cautionary note to this issue is that the experimental design and methodology used to study fatty acid transport varies considerably and may account for some of the measured differences between systems. Recent studies, however, using a dual-fluorescence approach to monitor uptake of cis-parinaric acid simultaneously with changes in the intracellular pH in adipocytes indicates that diffusion-mediated fatty acid uptake could be a significant even at low plasma LCFA_u concentrations [8]. Fatty acid transport in adipocytes is further complicated by the rapid rates of lipid metabolism and incorporation into the triglyceride droplet that enhance fatty acid uptake. It is likely that the rapid rates of fatty acid uptake observed in adipocytes may represent a balance between diffusion-mediated and protein-mediated processes.

PUTATIVE FATTY ACID TRANSPORTERS

Four proteins have been implicated by a variety of methods as functionally linked to fatty acid transport (Figure 1):

1. Plasma membrane fatty acid binding protein (FABPpm)
2. Fatty acid translocase (FAT/CD36)
3. Caveolin-1
4. Fatty acyl CoA synthetases (FATP and ACSL)

Plasma membrane fatty acid binding protein (FABPpm)

FABPpm is a 43-kDa protein expressed on the surface of various cells types such as adipocytes, hepatocytes, enterocytes and cardiomyocytes [9]. FABPpm was initially isolated by

oleate agarose affinity chromatography from solubilized rat hepatocyte plasma membranes and identified as a mitochondrial aspartate aminotransferase (mAspAT), a critical component of the malate-aspartate shuttle [10]. Evidence supporting the role of FABPpm in adipocyte fatty acid uptake came from the finding that anti-FABPpm antibodies selectively inhibited uptake of oleate in 3T3-L1 adipocyte monolayers without affecting 2-deoxyglucose or octanoate (medium-chain fatty acid) uptake [11]. Furthermore, expression of FABPpm in 3T3-L1 preadipocytes that normally do not express FABPpm caused a significant increase in fatty acid uptake.

The expression of FABPpm is regulated also supporting its role in FA uptake. FABPpm expression is altered in Zucker diabetic and obese rats and appears to correlate with uptake rates [12,13]. Prolonged activation of AMP-activated protein kinase by AICAR led to increase in expression levels as well as translocation of FABPpm from intracellular pools to the plasma membrane in cardiac myocytes [14]. Endurance training and fasting causes an increase in expression of FABPpm in muscle also coinciding with increased FA utilization. The mechanism by which FABPpm plays a role in LCFA uptake is unknown, although, it has been speculated that FABPpm and fatty acid translocase, another putative transporter may work in conjunction to increase LCFA uptake.

Fatty acid translocase (CD36)

CD36 is a transmembrane glycoprotein of apparent molecular mass of 88 kDa. CD36 is predicted to have two transmembrane domains at the N- and C-terminal, a large extracellular domain loop and two short intracellular cytoplasmic tails. CD36 belongs to a family of class B scavenger receptor and in addition to fatty acids is thought to bind a wide variety of hydrophobic molecules including thrombospondin, sickle cell erythrocytes, collagen, apoptotic cells and oxidized low-density lipoproteins [15,16]. CD36 is expressed in tissues with high FA metabolism rates such as adipose, heart and oxidative muscle [17] and has led to the suggestion that CD36 functions as a FFA receptor, although biophysical evidence for such a function remains to be demonstrated. Evidence supporting the role of CD36 in adipocyte fatty acid uptake came from loss and gain of function studies in mice. Transgenic CD36 over expressing mice were found to have less body fat and lower levels of serum triglyceride, fatty acids and cholesterol whereas, CD36 knockout mice had impaired fatty acid uptake in metabolic tissues including adipocytes and increased plasma fatty acid and triglyceride levels [18]. Knockdown of CD36 by RNAi in 3T3-L1 adipocytes resulted in a significant decrease in both basal and insulin-stimulated fatty acid uptake [19]. Expression of CD36 in fibroblasts induced expression of a saturable, high affinity, phloretin-sensitive component of FA uptake [20].

On the plasma membrane CD36 localizes to lipid microdomains (Figure 1) that are small platforms within cellular membranes that are detergent-resistant and rich in sphingolipids, cholesterol and caveolae [21]. In addition to the cell surface, CD36 is also localized to the endoplasmic reticulum, intracellular vesicles and mitochondria. Various stimuli such as insulin, activation of the forkhead transcription factor and activation by AMPK causes translocation of CD36 from intracellular stores to the plasma membrane thereby enhancing FA uptake [22,23]. It has been suggested that a small GTPase Rab1a and its effector protein maybe involved in the signaling pathway for CD36 trafficking [23]. Obesity, insulin and activators of peroxisome proliferators-activated receptors γ (PPAR γ) have been shown to increase CD36 mRNA levels. CD36 modification such as ubiquitination on lysines 469 and 472 in the C-terminal domain and palmitoylation at cysteines 3, 7, 464 and 466 have been shown to regulate its protein interactions, subcellular distribution, and turnover [24,25]. Ubiquitination on CD36 is inhibited by insulin and enhanced by FAs [26]. The mechanism by which CD36 plays a role in LCFA uptake is unknown. It has been speculated that CD36 may bind and internalize LCFA

by endocytosis or may bind LCFA and work in concert with FABPpm to provide a high concentration gradient of LCFA across the plasma membrane facilitating uptake by other fatty acid transporters.

Caveolin-1

Caveolae are specialized microdomains in the plasma membrane formed by the clustering of lipid raft domains [21]. Caveolins are essential structural proteins within caveolae and have been described as integral hairpin-like proteins facing the cytosol (Figure 1). The caveolin class of proteins consists of three isoforms 1-3, of which caveolin-1 and -2 are expressed in adipocytes. The adipocyte plasma membrane is rife with numerous caveolae that account for up to 25% of the plasma membrane surface and are known to be involved in important cellular transport processes such as endo- and transcytosis and signal transduction [27,28]. Caveolae have been shown to play a role in cholesterol transport [29]. However, the speculated role of caveolae in facilitating LCFA uptake is still unclear. Caveolin-1 has been shown to bind fatty acids [30]. Studies in HepG2 hepatoma cells indicate that fatty acids that are influxed into cells travel within a vesicular compartment that stained positive for caveolin-1 [31]. Furthermore, caveolin-1 was shown to move to the lipid droplet from the plasma membrane in response to free fatty acids [32]. Based on these observations, it has been speculated that fatty acids accumulate within the caveolar membrane, perhaps by binding to caveolin-1, leading to membrane asymmetry and budding of caveolae from the plasma membrane to form vesicles. These fatty acid loaded caveolae vesicles could deliver fatty acids to subcellular compartments for further metabolism. Caveolae have been shown to be associated with actin filaments forming a Cav-actin structure entailing caveolar vesicle transport to require an intact actin cytoskeleton [33].

However, it is unclear as to whether caveolin-1 plays a direct role in LCFA uptake or indirectly affects LCFA uptake by being structurally critical for caveolae formation and the correct localization and function of another fatty acid transporter CD36 [34]. Disruption of caveolar function by depletion of membrane cholesterol with cyclodextrin or breakdown of the actincaveolae structure with actin depolymerizing agents led to a decrease in LCFA uptake [33]. Furthermore, caveolin-1 null mice lack caveolae in adipocyte plasma membranes and exhibit severely elevated serum fatty acids and triglycerides, reduced adipocyte lipid droplet size and resistance to diet-induced obesity [35]. Recent studies using caveolin-1 knockout mouse embryonic fibroblast (Cav-1 KO MEFs) indicates that deficiency of caveolin-1 resulted in a complete loss of caveolae, absence of CD36 plasma membrane expression and a reduction in fatty acid uptake. Expression of caveolin-1 in cav-1 KO MEFs led to a reformation of caveolae, localization of CD36 to the plasma membrane and restoration of fatty acid uptake [34]. These studies support an indirect structural role of caveolin-1 in caveolae formation thereby controlling surface availability or stability of CD36 within the plasma membrane that is crucial to LCFA uptake.

Results from a study of LCFA uptake in HEK293 cells stably expressing caveolin-1 argues for a more direct role of caveolin-1. HEK293 cells do not express detectable amounts of caveolin-1 and other putative fatty acid transporters such as CD36 and Fatty acid transport protein-1. Increasing expression levels of caveolin-1 in HEK293 cells to threshold levels that mirror those in adipocytes led to a 2-fold increase in the transmembrane LCFA flip-flop rate [36]. A possible explanation being a direct role of caveolin-1 in LCFA influx by virtue of its function in binding and sequestering LCFA to the inner leaflet of the membrane bilayer. Interestingly, the alteration in LCFA transport was found to be independent of LCFA metabolism or expression of other putative LCFA transporters.

FATTY ACYL COA SYNTHETASES

Fatty acid transport proteins (FATP) and long-chain acyl-CoA synthetases (ACSL) are two different classes of membrane bound enzymes catalyzing the ATP-dependent esterification of long-chain (ACSL) and very long-chain (FATP) fatty acids to their acyl-CoA derivatives [37,38]. Proteins belonging to both classes of proteins have common ATP/AMP binding and fatty acid signature motifs. In mammals, six isoforms of FATP (FATP1-6) and five isoforms of ACSL (ACSL1, 3, 4, 5 and 6) have been identified with tissue specific expression patterns [39]. White adipose tissue predominantly express FATP1, FATP4 and ACSL1 while brown adipose tissue, in addition expresses ACSL5.

Fatty Acid Transport Protein 1

FATP1 was identified as being putatively involved in LCFA uptake by an expression-cloning screen in 3T3-L1 adipocytes [40]. Furthermore, the role of FATP1 in fatty acid uptake has been demonstrated by various loss of function/ gain of function model systems. Knockdown of FATP1 expression by RNAi in 3T3-L1 adipocytes as well as studies in primary adipocytes from knockout mice have indicated an absolute requirement for FATP1 in insulin-stimulated LCFA uptake [41]. Over expression of FATP1 in heterologous systems such as mouse heart and HEK 293 cells led to an increase in LCFA uptake [42,43].

FATP1 has a molecular mass of approximately 71 kDa and is thought to be an integral membrane protein. Membrane topology studies reveal a hydrophobic domain at the N-terminal that may be membrane anchored and other membrane associated domains that peripherally associate with the inner leaflet of the plasma membrane. Fractionation and immunochemical studies in 3T3-L1 adipocytes and in mouse primary adipocytes have revealed a variable pattern for the subcellular localization of FATP1. FATP1 was found in glycerolipid-rich non lipid raft region of the plasma membrane (Figure 1), high-density membranes (endoplasmic reticulum, golgi, mitochondria) and to a lesser amount in low density membranes (vesicles, endosomes) [44]. As with other putative fatty acid transporters, FATP1 was found to translocate to the plasma membrane from internal vesicles in 3T3-L1 adipocytes in response to insulin [44]. In mammalian 3T3-L1 adipocytes FATP1 was found to be capable of oligomerization, forming dimers and higher order structures [45].

The molecular mechanism by which FATP1 functions in LCFA uptake is still unknown. Evidence supports the requirement of acyl CoA synthetase activity of FATP1 and the generation of a FATP1 dimer to be crucial to its role in LCFA uptake. Loss of the acyl CoA synthetase activity of FATP1 due to a S250A mutation in the highly conserved AMP signature motif was shown to impair its function in fatty acid transport. Importantly, the mutation did not alter the ability of FATP1 to be expressed or trafficked to the plasma membrane [46]. Transient co-transfections of NIH 3T3 cells with wild-type FATP1 and increasing amounts of the S250A mutant was found to have a dominant negative effect on fatty acid transport. This indicates that the mutant could interfere with the generation of a functional FATP1 dimer crucial for its role in LCFA influx [45]. Based on the above evidence, a hypothetical scheme by which FATP1 functions in LCFA uptake could be devised. FATP1 could translocate to structurally disordered non-lipid raft regions of the plasma membrane in response to insulin. FATP1 could then abstract LCFAs from the inner membrane leaflet and esterify it to CoA thereby, preventing its efflux and driving a LCFA concentration gradient across the membrane. Thus, FATP1 may facilitate LCFA uptake by a process termed vectorial acylation, a mechanism of driving LCFA transport across the plasma membrane by tightly coupling it to metabolism. Experiments in 3T3-L1 adipocytes indicate that the vast majority of incoming fatty acids are converted into acyl-CoAs and preferentially shunted into triacylglycerol synthesis [41]. Ost et al. have reported that the conversion of incoming LCFA to triacylglycerol occurs on or around the plasma membrane in rat adipocytes [47]. Hence, it is plausible that a structured lipid

synthesis machinery including FATP1 may exist at the plasma membrane that mechanistically links fatty acid influx to triacylglycerol synthesis.

The expression of FATP1 is regulated supporting its role in LCFA uptake. The increase in adipocyte mRNA levels of FATP1 was found to correlate with the increase in fatty acid uptake in Zucker rat models of genetic obesity and non-insulin dependent diabetes [12]. Activators of PPAR γ were shown to induce transcription of FATP1 in adipose tissue coincided with the concomitant increase in LCFA uptake observed. Activators of retinoid X receptor (RXR), the heterodimeric PPAR nuclear receptor partner were also found to increase FATP1 gene transcription in 3T3-L1 adipocytes [48]. In addition to affecting translocation of FATP1, insulin was shown to negatively regulate FATP1 gene expression. Fasting and refeeding conditions that are known to alter serum insulin levels were found to induce and repress FATP1 gene transcription, respectively [49]. FATP1 transcription was also found to be down regulated by cAMP. Increased levels of cAMP increases catecholamine activation that was shown to enhance membrane transport of LCFA in rat adipocytes [50]. In addition, lipopolysaccharides and cytokines like TNF and IL-1 were shown to down regulate FATP1 mRNA levels in the adipocyte [51].

Fatty Acid Transport Protein 4

FATP4, a close homologue of FATP1 (~60% identity) is expressed in adipose tissue, skin, heart, skeletal muscle, liver, and is the only FATP isoform expressed in the small intestine where it was thought to function in intestinal lipid absorption [52,53]. The role of FATP4 in fatty acid uptake in heterologous systems has been demonstrated by various loss of function/gain of function model systems. FATP4 when over expressed in HEK 293 cells was shown to enhance fatty acid uptake, whereas incubation of isolated enterocytes with *FATP4*-targeted anti-sense nucleotides led to a 50% decrease in oleate and palmitate uptake [53]. However, in 3T3-L1 adipocytes, knockdown in expression levels of FATP4 by RNAi did not affect basal nor insulin-stimulated LCFA uptake [41]. Study of the role of FATP4 in LCFA uptake in mouse tissue was complicated by the fact that a total FATP4 gene disruption is lethal. The *Fatp4*^{-/-} null mice displayed features of neonatal lethal restrictive dermopathy, which is a rare human genetic disorder suggesting a critical function of FATP4 in the formation of a healthy epidermal barrier [54]. Transgenic expression of FATP4 from a keratinocyte-specific promoter rescued the perinatal lethality in FATP4 knockout. However, these mice do not display any defect in intestinal lipid absorption [55]. Localization of FATP4 is confined to the endoplasmic reticulum in fibroblast and COS cells and not to the plasma membrane as observed with other LCFA transporters [56]. Collectively, these studies imply that FATP4 does not play a role in LCFA uptake, although the physiological role of FATP4 is still unknown.

Acyl CoA Synthetase Long Chain 1

ACSL1 is a ~78 kDa membrane protein expressed in adipocytes and localized to various subcellular sites including the plasma membrane, high-density membranes, lipid droplets, and glucose transporter 4-containing vesicles [57-59]. ACSL1 was also identified as a putative LCFA transporter in the same genetic screen used to identify FATP1 as a LCFA transporter. In 3T3-L1 adipocytes ACSL1 was found to co-localize with FATP1 [58]. Additionally, over expression of ACSL1 in fibroblasts led to an increase in LCFA uptake [60]. The evidence thus supported a co-operative role in LCFA transport across the adipocyte plasma membrane. In contrast, knockdown of ACSL1 expression by RNAi in 3T3-L1 adipocytes indicated a role of ACSL1 in LCFA efflux and not influx. ACSL1 was found to be involved in the reacylation of LCFA released from the lipid droplet during basal and hormone-induced lipolysis. Acylation of LCFAs led to reincorporation into the triglyceride droplet thereby preventing its cellular efflux [19]. The proposed role of ACSL1 in adipocytes is similar to its role in rat primary

hepatocytes where LCFA were found to be channeled into diacylglycerol and phospholipids synthesis and reacylation into triglyceride synthesis [61].

CONCLUSIONS

Transport of LCFA across the adipocyte plasma membrane is a highly complex process. There is compelling evidence for the role of two different but not mutually exclusive processes: diffusion and protein-mediated uptake. It is likely that under cellular physiological conditions, LCFA influx observed may represent a balance between both processes.

Despite evidence for the role of putative fatty acid transporters in LCFA uptake, their molecular mechanism is still not very well understood. This, in part, could be due to overlapping roles of putative LCFA transporters in transport as well as cellular metabolism as opposed to the traditional role of transporters involved in glucose and ion-transport that are dedicated to uptake. It is also plausible that two or more transporters may work in concert to facilitate LCFA uptake. Cellular conditions that stimulate LCFA uptake can induce translocation of CD36 and FABPpm from intracellular depots to lipid raft-rich domains of the plasma membrane [Figure 1]. CD36 may assist in dissociation of LCFAs from serum albumin by binding LCFAs, and in conjunction with FABPpm facilitate transport or passive flip-flop of LCFA across the membrane bilayer. Consequently, caveolin-1 could bind LCFAs on the inner leaflet of the lipid bilayer and shuttle fatty acids via vesicle mediated transport to subcellular membrane compartments for further metabolism. Alternatively, FATP1 may play a major role in insulin-stimulated lipid-raft independent LCFA uptake in adipocytes. LCFA may be made available to FATP1 via 1) passive diffusion or 2) transport of LCFA across the lipid bilayer by CD36/FABPpm in lipid rafts and lateral diffusion in the inner leaflet to the less ordered non-lipid rafts region of the plasma membrane localizing FATP1. FATP1 could then esterify LCFA to CoA leading to their incorporation into the lipid droplet thus facilitating LCFA uptake by driving a LCFA gradient across the plasma membrane. In the future, examining these pathways to determine the impact of each will be important to understand how LCFA uptake and storage can be manipulated for therapeutic purposes.

REGULATION OF LIPOLYSIS

Adipocyte lipolysis encompasses the hydrolysis of triacylglycerol (TAG) and release of fatty acids for use as an energy source by other tissues such as the heart and skeletal muscle. The regulation of adipocyte lipolysis is an intricate balance of signaling cascades to release fatty acids during times of energy need. This process is highly regulated and as evidenced in obesity when mis-regulated can lead to insulin resistance. The present data suggests that regulation of lipolysis occurs mainly at the level of hydrolysis with little, if any, regulation of the efflux of fatty acids once they are hydrolyzed. Many different signals and signaling cascades participate in regulating the lipolytic machinery in adipocytes and thus an understanding of the enzymes involved is necessary for a complete understanding of the regulation of adipocyte lipolysis (Figure 2).

LIPASES

Complete hydrolysis of TAG results in three molecules of fatty acid and one molecule of glycerol. Three lipases have been implicated as the major enzymes of adipocyte lipolysis, adipose triglyceride lipase, also known as desnutrin (ATGL), hormone sensitive lipase (HSL) and monoacylglycerol lipase (MGL). The present data indicates that ATGL is the main triacylglycerol lipase, HSL is the main diacylglycerol (DAG) lipase and MGL is the main monoacylglycerol (MAG) lipase (Figure 2). Many other TAG lipases have been recently identified including triacylglycerol hydrolase, adiponutrin and GS2 yet their physiological roles in adipocyte lipolysis are unclear at this time [62-64].

Hormone Sensitive Lipase

Until recently, HSL was considered the rate-limiting enzyme in adipocyte lipolysis, hydrolyzing TAG and DAG while MGL was necessary for the hydrolysis of MAG [65]. HSL has several different isoforms with the predominate isoform being 84 kDa and is modeled to be composed of an N-terminal docking domain for fatty acid binding proteins and a C-terminal catalytic domain [66]. HSL exhibits triacylglycerol, diacylglycerol, retinyl ester and cholesterol ester hydrolase activity, having 10-fold more activity in vitro towards DAG than the other substrates [66]. HSL is a unique lipase in that it responds to stimulation by numerous hormones and metabolic signals. This response is primarily due to phosphorylation by PKA at three sites Ser563, Ser659 and Ser660 [67]. Mutation of Ser659 and Ser660 completely abolishes HSL stimulation of lipolysis in 3T3-L1 adipocytes [68]. HSL also has phosphorylation sites for AMPK at Ser565 and extracellular regulated kinase at Ser660 yet the physiological consequences for these are controversial or unclear respectively [69,70]. Knockout mice showed that while TAG hydrolysis was unchanged there is a massive accumulation of DAG, suggesting that HSL is not involved in TAG hydrolysis but is the main DAG lipase in adipose tissue [71-73]. Neutral cholesterol ester hydrolase activity was absent in the HSL KO confirming that it is the main cholesterol ester hydrolase in adipocytes. The KO studies suggested that other lipases played a major role in TAG hydrolysis that resulted in the finding of ATGL/desnudin by three independent groups [62,74,75].

Adipose Triglyceride Lipase

ATGL is a patatin domain containing enzyme which shows specificity towards TAG as a substrate. ATGL is a 54 kDa protein with a GXSSG motif, an α/β hydrolase fold, a conserved aspartate in the DX(G/A) motif and a glycine-rich region [74]. In vitro studies determined that ATGL is a TAG lipase and has 10-fold higher activity towards TAG compared to DAG [74]. Knockdown studies in 3T3-L1 adipocytes and global knockout of ATGL in mice revealed that it was involved in TAG hydrolysis and not DAG hydrolysis [75,76]. ATGL knockdown experiments in HSL null adipocytes showed that these two enzymes make up 95% of TAG lipase activity and are necessary for stimulated lipolysis [76]. These results suggest a synergistic relationship between the two lipases with ATGL hydrolyzing TAG and HSL hydrolyzing DAG.

Monoacylglycerol Lipase

MGL is an α/β hydrolase fold containing enzyme with specificity for MAG with little to no in vitro activity towards TAG or DAG [77]. Although HSL has in vitro MAG lipase activity MGL has been shown to be necessary for the hydrolysis of MAG in vivo [77]. It is highly abundant and thought not to be rate-limiting or play a role in the regulation of lipolysis.

LIPID DROPLET AND LIPID BINDING PROTEINS

Many other proteins play significant roles in regulating lipolysis in adipocytes. Lipid droplets are increasingly recognized as dynamic organelles, regulated by the proteins that coat them. Proteomic techniques have revealed numerous proteins which associate with lipid droplets, including structural proteins, small GTPases and signaling proteins [78]. One family of lipid droplet associated proteins, the perilipin family, including adipose differentiation related protein, TIP-47, S3-12 and Perilipin, is recognized as being essential to the storage and lipolysis of TAG (reviewed in [79-81]). The most well characterized member of this family, Perilipin protects the TAG from being hydrolyzed under basal conditions and then participates in the stimulation of lipolysis through the recruitment of lipases and the dispersion of the substrate TAG. Perilipin A and B are splice variants with Perilipin A being the most abundant and the most important in the regulation of lipolysis in adipocytes [82]. Perilipin A KO animals are resistant to diet induced obesity suggesting that it is a negative regulator of lipolysis [83].

Although it inhibits basal lipolysis it plays a significant and necessary role in stimulated lipolysis. Over expression of perilipin A results in the accumulation of TAG due to decreased lipolysis and not increased TAG synthesis [83,84]. This over expression also is necessary for stimulated lipolysis in heterologous cell lines that normally don't store TAG [85]. Perilipin A is phosphorylated at 6 sites by PKA [86,87]. Mutation of these sites results in the attenuation of stimulated lipolysis [85]. Ser-492 is necessary for the dispersion of lipid droplets after lipolytic stimulation while Ser 517 was shown to be necessary for full stimulation of lipolysis in 3T3-L1 adipocytes [88,89]. The consensus from studies of perilipin A is that it is necessary for the protection of lipid droplets under basal conditions while also being necessary for the stimulation of lipolysis through the actions of PKA. This puts perilipin at the center of the regulation of lipolysis (Figure 2).

An additional important lipid droplet associated protein is fat specific protein 27 (FSP27). Originally identified as a member of the cell death inducing DNA fragmentation factor- α -like effector family of proteins, more recent work has linked FSP27 to the regulation of droplet size and dynamics. Targeted disruption of FSP27 leads to smaller triacylglycerol droplets, increased lipolysis and enhanced mitochondrial activity while over expression promotes large droplets, reduced lipolysis and decreased mitochondrial activity leading to the conclusion that FSP27 may be a control factor in triacylglycerol dynamics [90,91].

CGI-58 was initially identified as the protein mutated in Charnin-Dorfman syndrome [92]. Charnin-Dorfman syndrome results in the ectopic storage of TAG in many tissue and specifically skin cells. CGI-58 is an α/β hydrolase fold containing protein that resembles a lipase but has no lipase activity due to having no active site serine [93]. CGI-58 interacts with Perilipin A at the lipid droplet surface under basal conditions and dissociates under PKA activation [94]. Recently CGI-58 was shown to be a co-factor for ATGL [93]. It is necessary for ATGL activity and shows no co-factor activity towards HSL. The increased activity of ATGL in the presence of CGI-58 is correlated with interaction between the two proteins. Indeed it has been shown that under PKA stimulated conditions ATGL and CGI-58 form a complex suggesting that this is the mechanism for the role of ATGL in stimulated lipolysis [95].

Another player in lipolysis is adipocyte fatty acid binding protein (AFABP). AFABP KO animals exhibit decreased fatty acid efflux under basal and stimulated conditions with an accumulation of intracellular fatty acids, suggesting that the protein is necessary for trafficking fatty acids to the membrane for efflux [96]. As fatty acids are negative regulators of HSL this process may result in increased lipolysis. AFABP interacts with phosphorylated HSL in a fatty acid dependent manner, suggesting it may also feedback inhibit HSL or alter other HSL interactions in the face of increased levels of fatty acids [97-100]. Although the significance of the interaction in vivo is unclear, AFABP is clearly involved in the efflux of fatty acids from the cell in an interaction independent manner. Overall, AFABP is considered to facilitate the efflux of FFA from the droplet surface to the plasma membrane.

β -ADRENERGIC RECEPTORS IN HUMANS AND MICE AND THEIR INFLUENCE ON LIPOLYSIS

The main physiological pathway for the activation of lipolysis is by catecholamines. Catecholamines interact with adrenergic receptors to give rise to intracellular signaling and functional outcomes. Adrenergic receptors are seven transmembrane G-protein coupled receptors. β -1, β -2 and β -3 adrenergic receptors are couple to $G_{\alpha s}$ which stimulate adenylyl cyclase (AC) activity while α 1 and α 2 adrenergic receptors are coupled to $G_{\alpha i}$ which inhibits AC activity [101]. As the same signaling molecule binds to these receptors it is the affinity and the abundance of each that alters the activity of AC. The β -receptors have a lower affinity for catecholamines than the α -receptors, thus in the basal state when catecholamine levels are low

AC activity will be low. At high concentrations of catecholamines the β -receptors become active and because of increased abundance compared to the α -receptors this favors increased AC activity. In mice the β_3 -receptor is the major regulator of lipolysis while in humans β_3 plays no role in stimulated lipolysis [101]. In humans, β_1 and β_2 provide the stimulating side while α_2 controls the inhibitory side [101]. This balance of AC activity is the main mechanism regulating adipocyte lipolysis.

STIMULATION OF LIPOLYSIS

The main signaling mechanism stimulating adipocyte lipolysis is through G_{α_s} coupled receptors activating AC as discussed above. AC converts ATP to cyclic-AMP (cAMP) that acts as a second messenger. The regulatory subunit of PKA binds cAMP and dissociates from the catalytic subunits thereby activating the kinase. PKA then phosphorylates at least two important downstream targets, HSL and perilipin. Upon phosphorylation of HSL at Ser659 and Ser660 it translocates to the lipid droplet surface and interacts with Perilipin A [102, 103]. Perilipin A phosphorylation at 6 sites results in the dispersion of lipid droplets to smaller droplets, is necessary for HSL translocation and interaction, and as discussed above inhibits its interaction with CGI-58 [94,102,103]. Ser517 phosphorylation of Perilipin A is necessary for stimulated lipolysis, suggesting that it is the main intracellular governor of lipolysis [89]. Once released from Perilipin A, CGI-58 interacts with ATGL which increases ATGL activity and allows it to associate with the lipid droplet [93]. Thus HSL and ATGL have increased activity and substrate access resulting in the increased hydrolysis of TAG and DAG.

Catecholamines are the main physiological agent which stimulates lipolysis through this pathway. Glucagon produced by the pancreas is secreted during long term fasting and also stimulates through a G-protein coupled receptor to stimulate lipolysis. Many hormones and cytokines have been implicated in the stimulation of lipolysis such as growth hormone, IL-6, prolactin, and parathyroid hormone. The physiological impact of these is yet to be determined and depending on the organism studied there is varying effects. The mechanisms of activation of lipolysis tend to be in the fine tuning of catecholamine induced lipolysis through the up-regulation of β -adrenergic receptors or the down-regulation of α -adrenergic receptors.

INHIBITION OF LIPOLYSIS

As discussed above, inhibition of lipolysis can occur through increasing G_{α_i} coupled receptor activation leading to inhibition of AC. Adenosine, prostaglandin E2 (PGE2) and NPY are examples of molecules that inhibit lipolysis in this manner. Stimulation of the A1-adenosine receptor, NPY-Y1 receptor and EP3 receptor results in the inhibition of lipolysis while antagonists to these receptors enhance lipolysis suggesting that they may have a role in fine tuning the lipolytic response [104-106]. In addition, PGE2 has been implicated as an important regulator of lipolysis in vivo. Global knockout of a novel phospholipase, AdPLA results in increased lipolysis [107]. The mechanism of action was shown to be due to a decrease in PGE2 resulting in increased cAMP levels. This study highlights PGE2 as a major regulator of adipocyte metabolism in an autocrine/paracrine fashion. Long-chain fatty acids, β -hydroxybutyrate and L-lactate have all been implicated as negative regulators of lipolysis through a G_{α_i} linked receptor. These G_{α_i} signaling compounds may be important autocrine/paracrine regulators of lipolysis under different metabolic states fine-tuning the lipolytic machinery depending on the body needs.

The main physiological hormone involved in the negative regulation of lipolysis is insulin. Insulin signals through its receptor tyrosine kinase to recruit insulin receptor substrate 1 which is phosphorylated and recruits downstream signaling molecules. One of those is the PI3 kinase that converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate. AKT/PKB is recruited to the membrane through its pleckstrin homology domain

and is subsequently phosphorylated and activated by PDK1. Activated AKT/PKB then phosphorylates two important targets for the down regulation of lipolysis. First is phosphodiesterase 3B which hydrolyzes cAMP to form 5'AMP and thus decreases PKA activation [108]. The second is protein phosphatase 2A, 2C and 1 which then dephosphorylates HSL and perilipin decreasing lipolysis [109]. Recently the insulin signaling through FOXO was shown to inhibit ATGL mRNA production [110,111]. This pathway may be important to the increased basal lipolysis seen in insulin resistant animals. The insulin signaling pathway is thought to be the mechanism of decreased lipolysis after feeding and a major portion to the anabolic effects of insulin.

ALTERNATE LIPOLYTIC PATHWAYS

Natriuretic peptides

A human specific pathway to stimulate lipolysis has recently been identified. Natriuretic peptides bind to their guanylyl cyclase receptors increasing cGMP which activates protein kinase G (PKG) resulting in the phosphorylation and translocation of HSL to increase lipolysis [112]. Confounding this mechanism is the fact that cAMP levels also rise and PKA is activated making it unclear if PKG or PKA phosphorylates HSL. In the end this is a potent stimulator of human adipocyte lipolysis.

TNF α

An alternate regulation pathway for lipolysis is through TNF α . TNF α is a classical inflammatory cytokine resulting in insulin resistance in adipocytes. TNF α signals through its receptor TNF receptor 1 in adipocytes, that activates extracellular regulated kinase (ERK) and c-jun N-terminal kinase (JNK) to affect transcriptional programming of the cell important for lipolysis [113]. Three mechanisms dominate the TNF α induced lipolysis. Firstly, TNF α inhibits insulin signaling through the serine phosphorylation of IRS-1 thus limiting the tyrosine phosphorylation terminating the signal [114,115]. This pathway will result in increasing lipolysis in the face of increased insulin. Secondly, TNF α down regulates all forms of G α i in rodent adipocytes thus increasing AC activity and lipolysis [116]. This pathway is not seen in human cells treated with TNF α . Thirdly, ERK and JNK down regulate perilipin A in a direct manner and indirectly increase perilipin A phosphorylation. The increased phosphorylation is due to decreased PDE3B transcription by ERK resulting in increased cAMP levels and PKA activation [117]. Down regulation of perilipin by TNF α through ERK and JNK results in increased basal lipolysis and decreased stimulated lipolysis [113,118]. The down regulation of Perilipin A is the major determinant of increased lipolysis with TNF α exposure. TNF α stimulated lipolysis is a delayed response compared to β -adrenergic signaling, taking on the order of 6 hours to increase lipolysis and 48 hours for maximal activation. This suggests that TNF α is a major determinant of the tone of adipocyte lipolysis. In obesity, basal lipolysis is increased while stimulated lipolysis is unchanged or diminished. At the same time TNF α levels are increased and may, through the down regulation of perilipin, be the reason for the obesity linked increase in basal lipolysis.

CONCLUSIONS

The regulation of lipolysis is a balancing act between numerous signals and downstream effectors. This balancing act is in place to store TAG in times of excess energy while being able to rapidly mobilize this high energy substrate during times of energy need. This process is regulated by the central nervous system, hormones dictating the energy state of the body, such as insulin and glucagon, as well as autocrine/paracrine factors which allows for depot specific changes depending on the metabolic status of the tissue. This interplay is unbalanced in obesity and results in the accumulation of plasma fatty acids which alters other tissues insulin

responsiveness and metabolism. Understanding the mechanisms of lipolytic regulation is key to determining targets for therapeutic approaches to this growing epidemic. Although many lipolytic pathways are known today the physiologic impact of all of these is unclear. Going forward, understanding these pathways in depth and discovering yet identified pathways and modulators will be of great use in the treatment of obesity and insulin resistance.

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Figure 1.

Schematic representation of free fatty acid (FFA) influx mediated by binding to CD36 in caveolin-1-FABP_{pm} rich microdomains. In one possible scenario, FFA diffuse laterally in the plasma membrane and flip flop across to the inner leaflet where they are enzymatically esterified by FATP1 in an ATP and CoA dependent manner.

Figure 2.

Diagrammatic representation of regulated lipolysis. In this scheme, protein kinase A (PKA) dependent phosphorylation of hormone sensitive lipase leads to its translocation from the cytosol to the surface of the lipid droplet. Concurrently, PKA dependent phosphorylation of perilipin A leads to its degradation and loss from the droplet surface enabling assembly of a functional lipolysis complex. Such an activated complex facilitates complete hydrolysis of TAG to glycerol and FFA.