Review

The role of lipases and autophagy in lipolysis

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Abstract

The balance of lipid storage and utilization of lipids as a substrate of energy is maintained by complex biological signals. During nutrient-deficient condition or excess physical activity, fatty acids are produced through lipolysis of lipid droplets by lipases and provide energy, or autophagy is induced. This review will summarize the regulators of lipolysis that maintain energy homeostasis and cellular signaling.

Introduction

Body fuel selection of either glucose or fatty acids is altered in the complexities of dietary composition, age, disease, and various stresses to sustain energy homeostasis by using hormones and enzymes. Fatty acids are stored as triacylglycerol in adipose tissue constituted normally with amounts to 15–20% of body weight. Food intake and energy balance play the most important role on controlling fat mass. According to WHO reports, obese individuals with BMI greater than or equal to 30 is about 13% of the world's adult population (11% of men and 15% of women) in 2014. Obesity or overweight (BMI greater than or equal to 25) is a major risk for cardiovascular diseases (mainly heart disease and stroke), diabetes, musculoskeletal disorders (especially osteoarthritis), and some cancers (endometrial, breast, and colon). Increasing incidences of both obesity and diabetes make the research on metabolism of adipose tissue progressive.

Mammals can physiologically control the balance between lipolysis and lipogenesis to maintain cellular energy homeostasis. The control of energy balance is critical to survive in various environment, such as starvation, cold temperature, and hypoxia. The typical example is that the amount of adipose tissue is influenced by surround environments. For an example during hibernation, being near 0.7 of the respiratory quotient (RQ; the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed) indicates that fat is the major source for energy production to activate fatty acid metabolism during hibernation. The production of fatty acids from lipid stored in adipose tissue requires lipolytic enzymes. This review will focus on the activity of lipases that are biological adipose markers particular in lipolysis. In addition to lipases, it will describe the autophagy on adipose tissue and other tissues in the interaction to lipase. We summarize the mechanisms and regulators of lipase and autophagy which are key steps to maintain energy homeostasis and cellular signaling.

1. Enzymes related to Lipolysis

Mammals store energy in white adipose tissues as triglycerides. During food intake, an excess of dietary fatty acids is esterified to triacylglycerol (TAG) and stored in cytosolic lipid droplet (LD) of adipocyte. During fasting or excess exercising, these TAG stored in LD are hydrolyzed in a process of lipolysis. Fatty acids degraded from TAG are delivered to peripheral tissues to produce ATP production by β-oxidation. Fatty acid is also essential substrates for the synthesis of lipids, such as membrane lipids and lipids involved in cellular signaling.

Lipolysis is the biochemical pathway of the catabolism of triacylglycerol stored in cellular lipid deposits to produce free fatty acids. The mobilization of stored fats is mainly mediated by three kinds of lipolytic enzymes. First, triacylglycerol (TG) is selectively converted to diacylglycerol (DAG) by adipose triglyceride lipase (ATGL). Second, hormone-sensitive lipase (HSL) hydrolyzes DAG and TG to monoacylglycerol (MAG). Finally, monoacylglycerol lipase (MGL) cleaves MAG into glycerol and free fatty acids.

2. Adipose triacylglyceride lipase (ATGL)

2.1. The feature of ATGL

Hormone-sensitive lipase (HSL) had been thought as a major lipolytic enzyme. However, HSL- deficient mice did not show the increase of fat deposition and WAT mass and obesity.[1] These findings suggested the present of another key enzyme of lipolysis. In 2004, ATGL, known as TAG hydrolases, desnutrin, and patatin-like phospholipase domain containing protein 2 (PNPLA2), was identified as a lipase which catalyzes the initial step in triglyceride hydrolysis.[2-4] ATGL hydrolyzes TG to generate DAG and free fatty acids and also produces TAG and MAG from two DAG molecules in an acyl-CoA independent matter.[4] On these reactions, ATGL is a rate-limiting enzyme in free fatty acid mobilization. HSL is rapidly and dramatically up-regulated by lipolytic hormones such as catecholamines through PKA phosphorylation of perilipin but ATGL is independent of PKA phosphorylation.

ATGL is expressed at high levels in WAT and BAT and at low levels in testis, cardiac muscle, and skeletal muscle.[2] ATGL (PNPLA2 and Pnpla2) encodes 504 and 486 amino acid in human and mouse, respectively. ATGL contains an N-terminal patatin-like domain[5] which was originally discovered in lipid acyl hydrolase of potato and other plants.[3,6] The patatin domain in both the murine and the human enzyme contains a predicted esterase of the α/β hydrolase fold domain and can catalyze transacylation reactions independent of ATP and acyl-CoA. ATGL has Ser-47 and Asp-166 active sites which are both critical for TAG hydrolysis within the patatin domain in N-terminal.[6] The C-terminal half of ATGL is expected to consist mostly of α-helical and loop regions. It is thought that a hydrophobic stretch (315-360 amino acid) represents a binding region of lipid and effects enzyme activity and LD binding.[7,8]

2.2. The regulation factors of ATGL

Having lipolytic enzyme activity on lipid droplets, ATGL is transferred to an adequate localization with its coactivator. To increase hydrolase activity, ATGL requires a coactivator protein called comparative gene identification-58 (CGI-58, Abhd5) which is an α/β hydrolase fold enzyme. CGI-58 depends on PKA-dependent phosphorylation of perilipin.[9,10] Perilipin A (perilipin 1, lipid droplet-associated protein) is a lipid droplet scaffold and plays a key role in the regulation of hormone-stimulated lipolysis.[11-13] Perilipin activation leads to perilipin phosphorylation and release of CGI-58. Perilipin A, a predominant perilipin isoform in adipocytes, regulates ATGL-dependent lipolysis.[14] Both Ser-492 and Ser-517 of perilipin are shown as the PKA phosphorylation site. These sites are necessary for release of CGI-58 and interaction between CGI-58 and ATGL by β-adrenergic stimulation of PKA.[15]

Gene expression of ATGL mRNA is elevated by peroxisome proliferator-activated receptor (PPAR) agonist, glucocorticoids,[16] and fasting[3,17]. ATGL is a target for transcription by PPAR gamma.[18] SIRT1 upregulates expression of ATGL through PPAR gamma activity.[19] Forkhead homeobox type O1 (FoxO1) plays an important role in the control of insulin-dependent expression of ATGL.[20]

ATGL mRNA is down-regulated by the treatment of insulin, isoproterenol, or TNF alpha and by the induction of G coupled pathways by forskolin and cholera toxin in 3T3-L1 adipocytes.[21,22]. Activation of target of rapamycin complex 1 (TORC1) signaling inhibits expression of ATGL mRNA.[23] Although isoproterenol and TNF alpha

reduce ATGL mRNA levels in adipocytes, they stimulate lipase activities to release fatty acids and glycerol. This difference between enzyme mRNA levels and activities is explained by extensive posttranslational regulation of ATGL and HSL. Therefore, cellular lipase mRNA levels cannot evaluate enzyme activities.

The G0/G1 switch gene 2 (G0S2) induces cell cycle progression from the G0 to G1 phase of the cell cycle. G(0)/G(1) switch gene 2 (G0S2)-encoded protein binds directly to ATGL and inhibits ATGL-induced lipolysis.[24] G0S2 mRNA is the highest level in WAT, BAT, and liver and its encoded protein directly interacts with patatin-like domain of ATGL.[24] This interaction is independent of presence of CGI-58.[25] G0S2 mRNA level is upregulated by PPAR γ agonists in adipocytes[26], and by glucose in liver[27]. During adipogenesis, G0S2 mRNA level is upregulated in mice and human adipocyte cell.[26] The level of G0S2 is decreased by chronic high fat feeding and increased by treatment of insulin in WAT. On the other hand, the expression of G0S2 is increased by fasting and by treatment of PPAR gamma agonist in hepatic cell.[26]

In this way, the regulation of ATGL is related to adipocyte differentiation with energy homeostasis.

2.3. Functional linkage of ATGL

Since the energy homeostasis is executed on the interaction between the metabolism of carbohydrates and fatty acids, ATGL is interacted with the metabolic factors of carbohydrates. ATGL binds pigment epithelium derived factor (PEDF) which is a 50 kDa secreted glycoprotein and a regulator of TAG metabolism in liver. PEDK is reported that it has a role in promoting lipolysis through binding to ATGL in adipose tissue, muscle, and liver.[28-30] PEDF is inversely regulated by insulin and hypoxia. PEDK is up-regulated in patients with type 2 diabetes. The up-regulation of PEDF induces insulin resistance in adipocytes.[31] Fat-specific protein 27 (FSP27) is a differentiation-regulated protein in adipocytes. FSP27 interacts with ATGL to inhibit lipolysis in human adipocytes.[32] FSP27 regulates TG accumulation in ATGL dependent manner. Insulin acutely inhibits lipolysis. The decreased ATGL-derived FFAs leads to increase of glucose use, increase of glucose tolerance, and increase of insulin sensitivity.[33]

The deficiency of ATGL and CGI-58 induces neutral lipid storage disease. While CGI-58 mutations are associated with Chanarin-Doefman syndrome (ichthyosis), disease characterized by the presence of a large number of cytosolic lipid droplet containing TG in various tissues such as the skin, liver, and leukocytes,[34,35] mutations in the PNPLA2 were only reported with NLSD with myopathy (NLSDM).[36] Also, NLSDM shows the severe defect in triglyceride degradation in fibroblasts and the marked triglyceride storage in liver, muscles, and other visceral cells.[36,37] In cardiomyopathy, the lipolysis of triglycerides by ATGL is an essential factor related to the generation of lipid ligands for PPAR activation in cardiac muscle.[38]

3. Hormone-sensitive lipase (HSL)

3.1. The feature of HSL

HSL is an intracellular neutral lipase and is a rate-limiting lipase for the catabolism of DG. HSL-deficient mice accumulate DGs in adipose tissue.[39] Thus, it is known that HSL is more important to DG hydrolase than TG hydrolase. The phosphorylation state of HSL is changed to modulate free fatty acid secretion from adipocyte cells by hormones such as catecholamines, adrenocorticotropic hormone (ACTH), insulin, and natriuretic peptides.

The expression of HSL mRNA is observed in both WAT and BAT. Low HSL expression is observed in many other tissues and cells such as steroidogenic cells, muscle, testis, pancreatic β cells, and macrophages.[40,41] The characteristics of mRNA, protein size, and multiple potential transcription factor binding elements upstream of each exon of HSL are different in each tissues.[42,43] Therefore, it suggests the possibility of differential transcriptional regulation of HSL in different tissues and under various physiological conditions.

HSL has the 757-amino acid sequence. N-terminal region (1-300 amino acids) of HSL is a domain for interaction of HSL with adipocyte-binding protein (ALBP) and provides an additional mechanism for control of HSL activity.[44] C-terminal domain (307-768 amino acids) contains the α/β hydrolase fold which is a common structural fold in many lipases and esterases. Ser-424, Asp-693, and His-723 are reported as the catalytic domain.[45] Also, it is reported that Asp-703 and His-733 are related to lipase and esterase activity.[46] Moreover, the third region (Ser-563, Ser-659, Ser-660, and Ser-600) was found as a regulatory module

of enzyme through phosphorylation and activation by PKA and extracellular signal regulated kinase (ERK).[47-49] [Especially, Ser-563 is identified as markedly hydrophilic domain and a lipid-binding consensus site.[50]]

3.2. The regulation factors of HSL

The phosphorylation state of HSL determines the activity of lipolytic enzyme. HSL is phosphorylated by cyclic AMP-dependent protein kinase (AMPK) and a ubiquitous serine/threonine protein kinase activated in response to environmental or nutritional stress factors which deplete intracellular ATP levels.[51] The lipolysis by HSL is down-regulated by insulin, isoproterenol, and TNF α (which down-regulate ATGL in adipocyte[21]) though cyclic AMP-mediated dephosphorylation.[49] PKA activation promotes the translocation of HSL to lipid, which correlates with the magnitude of stimulated lipolysis in adipose tissue and muscle.[52,53] Perilipin A interacts with HSL and stimulates lipolysis in adipocytes.[14,54] It is reported that receptor-interacting protein 140 (RIP140) interacts with perilipin and controls lipolysis in adipocyte.[55] HSL rapidly and specifically translocates to lipid droplets (LDs) containing perilipin. This translocation is partially dependent on perilipin phosphorylation.[10] Although AMPK mediates HSL activity in adipose tissue[56], AMPK is expected to be a more important mediator of HSL activity than in adipose tissue. AMPK decreases HSL activity increased by exercise through attenuating β-adrenergic stimulation in muscle.[57] Extracellular signal-regulated kinase (ERK) enhances lipolysis activation of HSL by phosphorylating on Ser-600 amino acid.[48] Glycogen synthase kinase-4 and Ca2+/calmodulin-dependent kinase phosphorylate HSL at site 2 in vitro and in vivo.[58,59]

3.3. Functional linkage of HSL

HSL interacts with adipocyte lipid-binding protein (ALBP) which is a member of the family of intracellular lipid binding protein.[44] This interaction of HSL and ALBP suggests that trafficking of fatty acid generated during lipolysis is facilitated by ALBP.

HSL interacts with steroidogenic acute regulatory protein (StAR). Steroidogenic acute regulatory protein (StAR) delivers cholesterol to the inner mitochondrial membrane. Steroid hormones are synthesized from cholesterol which is mostly supplied by the selective uptake of lipoprotein-derived cholesteryl esters. HSL -/- mice have profound effects on cellular cholesterol homeostasis. HSL interacts the steroid hormone production on utilizing LDL-derived cholesteryl esters, as well as HDL-derived cholesteryl esters. Therefore, HSL facilitates cholesterol movement from lipid droplets to mitochondria for steroid hormone production.[60]

4. Monoacylglycerol lipase (MGL)

4.1. The function of MGL

MGL is a rate-limiting enzyme for the break-down of MGs derived from, extracellular TG hydrolysis, intracellular TG hydrolysis and intracellular phospholipid hydrolysis. MGL degrades MG to glycerol and fatty acid. MSL is one of the superfamily of α / β hydrolase fold protein with GXSXG motif. N-terminal region has catalytic triads of Ser-122, Asp-239, and His-269.[61]

4.2. The regulation factors of MGL

MGL mRNA is expressed in adipose tissue and various tissues, such as adrenal grand, ovary, heart, brain, lung, liver, skeletal muscle, kidney, and testis.[61] It indicates that MGL is a widespread intracellular monoglyceride-hydrolyzing enzyme.

4.3. Functional linkage of MGL

MSL inactivates endocannabinoid through hydrolysis of monoglyceride 2-arachidonoylglycerol (2-AG), one of activators of cannabinoid receptors in brain.[62,63] These findings suggest that MGL has the possibility of the physiological role in endocannabioid metabolism related to appetite, pain sensation, and mood control.

5. Autophagy

During starvation, the autophagy induced is believed to be a critical factor for energy homeostasis because a role of autophagy is energy recycling. The inhibition of autophagy increases TG and LD in vitro and in vivo.[64] Autophagy releases lipids and amino acid and uses as an energy source. The regulatory and functional mechanism of lipid release is similar to lipolysis. Thus, it is thought that the capability of lysosomes to degrade lipids may contribute to LD and TG breakdown in starvation.[64] The studies using hepatocytes and liver have demonstrated that autophagy of LD is required for fasting-induced lipolysis in murine liver and cultured

hepatocytes.[64] This study proposes the recruitment of microtubule-associated protein 1 light chain 3 (LC-3) on LD. LC-3 initiates formation of a limiting membrane though autophagy related protein 7 (Atg7)- dependent conjugation.[64] It is reported that hepatic Atg7 expression is decreased in ob/ob mice liver.[65] Adipose-specific knockout of Atg7 generated lean mice with reduced adipose mass, enhanced insulin sensitivity, and elevated rate of β-oxidation.[66] While adipose-specific Atg7-deficient mice show reduced WAT mass, BAT mass increases.[66,67] The function of autophagy in regulating adipose physiology[66,68] is quite different from the role of autophagy in mobilizing lipids in other cell types[69-71]. Therefore, in WAT, autophagy may play a role in the induction of adipocyte differentiation and lipogenesis but not in lipolysis. In relationship between the cellular recycling process of autophagy and longevity, autophagy and lipase Lipl-4 function is requested to ensure prolonged life span in *C. elegans*.[72]

Lysosomal lipase is called as "acid lipase" and thought to serve mainly in degradation of lipids contributed by the diet through endocytosis or those present in the membranes of organelles digested during the autophagic process. Lysosomal acid lipase (LAL, acid cholesteryl ester hydrolase) plays a role in intracellular hydrolysis of TG and cholesteryl esters. The released free cholesterol regulates endogenous synthesis of cholesterol, uptake of low density lipoprotein, and cholesterol esterification. Deficiency of LAL occurs in two autosomal recessive storage disorders, cholesteryl ester storage disease (CESD) and Wolman disease. The LAL gene expression is controlled by a transcription factor, forkhead homeobox type O1 (FoxO1). FoxO1 is a critical mediator both in the cellular stress pathway and in nutrient-regulated processes.[73] FoxO1 up-regulated under nutrient restriction modulates LAL gene expression in adipocytes[74]. FoxO1 also modulates lipid metabolism in adipose tissue by changing adipocyte size and up-regulating adipose tissue specific gene such as ATGL.

In the future

ATGL, HSL, and MGL are crucial lipases in the degradation from TG to glycerol and fatty acids (Fig. 1). Moreover, the interaction between lipases and autophagy may be an essential factor of metabolism of fat. The productive control of lipases is accurately synchronized with cell signals such as cellular function, differentiation, proliferation, and cell death for the correspondence to environmental stress. The lipolysis always produces fatty acid, TG, and cholesterol to reduce the adipose size or LDs. Lipases are linked to cellular functions excluding lipid metabolism. It is important which pathway of lipolysis is involved in the production of fatty acids

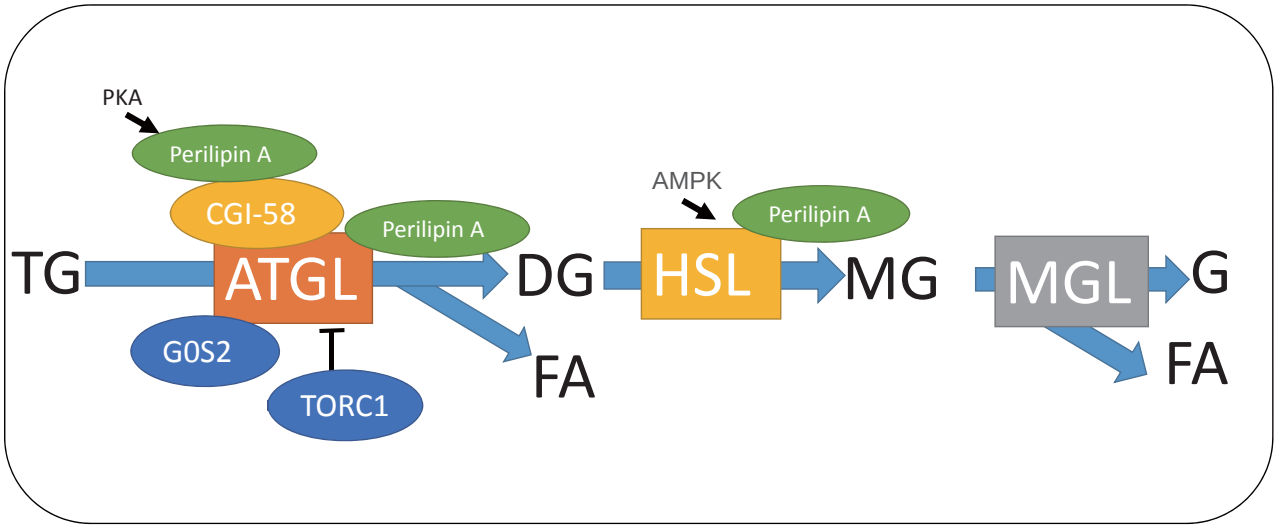

Fig1. The mechanism of lipolysis by ATGL, HSL, and MG in adipose tissue
TG(tryacylglycerol) is hydrolyzed by 3 lipase into G(glycerol) and FA(fatty acid). ATGL is a first rate-limiting enzyme of metabolism of TG. CGI-58 is a coactivator of ATGL. ATGL and CGI-58 are activated by perilipin A and lipolysis TG into DG and FA. G0S2 and TORC1 signaling inhibit the lipolysis activity of ATGL. HSL is a second rate-limiting enzyme of lipolysis. HSL is phosohrylated by AMPK. And perilipin interacts with HSL and promotes lipolysis. MGL cleaves MG(monoacylglycerol) into G and FA.

rather than the quantity of released fatty acid. In the future, main issues in lipid researches may not be to understand lipid metabolism just in energy homeoatasis but to understand the integration of cellular functions and energy homeostasis in its various patterns.

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