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Review article

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THE SKELETAL AND HEART MUSCLE TRIACYLGLYCEROL LIPOLYSIS REVISITED

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For 40 years, the enzyme hormone sensitive lipase was considered to hydrolyze the first ester bond of the triacylglycerol moiety and thus initiate hydrolysis. However, 12 years ago a new lipolytic enzyme, termed adipose triglyceride lipase was discovered. It was further shown that the process of lipolysis of triacylglycerol to diacylglycerol and fatty acid is initiated by adipose triglyceride lipase and not by hormone sensitive lipase, responsible for hydrolysis of diacylglycerol to monoacyglycerol and fatty acid. Adipose triglyceride lipase is present in all types of cells containing neutral fat. The enzyme is activated by a protein called comparative gene identification-58 and inhibited by a protein called G0/G1 switch protein 2. It has also been discovered that perilipins, the main proteins coating lipid droplets in the cells, are involved in the process of triacylglycerol lipolysis. Five perilipins (1-5) were identified, however, up to now their role has been poorly assessed. In skeletal muscles, exercise and training affect the mRNA expression and protein content of adipose triglyceride lipase, comparative gene identification-58, G0/G1 switch protein 2, perilipin 2 and 5. The effect of exercise/training depends on exercise intensity and type of muscle fiber. An interaction between comparative gene identification-58 and adipose triglyceride lipase seems to be responsible for the enzyme activation during contractile activity. Adipose triglyceride lipase is also responsible for the activation of the first step of triacylglycerol lipolysis in the heart. There is substantial evidence that cardiac triacylglycerol metabolism affects the function of the heart. ATGL gene mutations leads to the development of neutral lipid storage diseases.

Key words: *adipose triglyceride lipase, hormone sensitive lipase, comparative gene identification-58, G0/G1 switch protein 2, perilipins*

INTRODUCTION

Unesterified (free) long chain fatty acids (FA) play a crucial role as energy source for most cells of the body. They are also components of complex lipids, mostly phospholipids and triacylglycerol, and serve as precursors of different mediators. FA are esterified to glycerol backbone to form triacylglycerol (TG) and are stored in this form. One TG moiety contains three FA residues. Adipose tissue is a 'natural' store of TG. Only adipocytes release FA to the blood and thus supply other cells of the body with the compounds. TG are also present, although only in small amounts, nearly in each cell type. They are hydrolyzed in the process called lipolysis. Studies of the last 12 years have revolutionized our knowledge on the regulation of TG lipolysis. Several excellent reviews are devoted to the current view on TG metabolism in adipocytes (1-5).

The present contribution is devoted to the lipolysis of TG located in the skeletal and heart muscles. Regarding skeletal muscles, special attention will be paid to a relationship between the contractile activity and behavior of particular components of the lipolytic system. In the case of the heart, the relationship between cardiac TG metabolism and the function of the heart will be emphasized. First, brief information will be provided on lipolysis regulation in adipose tissue.

OVERVIEW OF TRIACYLGLYCEROL LIPOLYSIS IN ADIPOSE TISSUE

Triacylglycerol are stored in lipid droplets (LD). The white adipose tissue adipocyte usually contains one LD, whereas the brown adipose tissue adipocyte has several droplets. The size of the white adipocyte LD is around 100 μm (6). A lipid droplet contains TG and cholesteryl esters inside and is surrounded by a coat composed of a monolayer of phospholipids and different proteins. Perilipins are the best characterized LD proteins (1, 7, 8). Lipid droplets are now recognized to be active organelles and not only passive stores of TG (8-11). The mechanism of LD formation still remains a matter of controversy (1). Our view on the process of lipolysis has changed dramatically over the last 12 years. Until the year 2004, the enzyme called hormone sensitive lipase (HSL) was commonly accepted as the principal, rate limiting enzyme in the process of TG lipolysis (12). HSL was described in 1964 and was considered responsible for the hydrolysis of TG to diacylglycerol (DG) and then hydrolysis of DG to monoacylglycerol (MG) (13). MG is hydrolyzed to glycerol and fatty acid (FA) by the enzyme monoacylglycerol lipase (14). However, in 2004 three independent groups of investigators discovered a new lipase which is presently called adipose triglyceride lipase (ATGL). It has also been proved that ATGL starts the process of lipolysis hydrolyzing the first ester bond of

TG to diacylglycerol (DG) and a fatty acid (15-17). It was further shown that HSL plays the principal role in the hydrolysis of DG to MG. ATGL has 10-fold higher substrate affinity for TG than for DG and only small or none affinity for other lipids: MG, cholesteryl- and retinyl esters (12, 16). The maximal affinity of HSL to TG, DG, MG, cholesteryl and retinyl esters is in the range of 1:10:1:4:2 (5, 12). ATGL and HSL are responsible for 90 – 95% of TG lipolysis in the mice tissue. This may suggest the presence of other enzymes involved in TG hydrolysis (5, 7, 18).

Adipose adipose triglyceride lipase mRNA is present in all tissues examined, although its expression in the adipose tissue is much higher than in the other tissues (5, 16). The lipolytic activity of ATGL increases markedly when it binds to an activator protein (a coactivator) termed comparative gene identification-58 (CGI-58) (19). CGI-58 is present in different tissues, including the skeletal and heart muscles. At basal conditions, ATGL, perilipin-1 (PLIN1, see below) and CGI-58 reside on the surface of a lipid droplet, whereas HSL is present in cytosol. Monoacylglycerol lipase is found both in cytosol and on the LD surface. Recently, a selective ATGL inhibitor has been discovered. It is a protein called G0/G1 switch protein 2 (G0S2). It binds to ATGL and inhibits its activity. Overexpression of G0/G2 reduces lipolysis, whereas its deletion augments the process (20). The discovery of ATGL and its role in the initiation of lipolysis dethroned HSL as the key enzyme in the process. However, HSL plays a crucial role in the activation of the second step of lipolysis, i.e. hydrolysis of DG to monoacylglycerol and a fatty acid. In HSL knock-out mice, DG accumulate in different tissues (21). Monoacylglycerol lipase mRNA is expressed virtually in all tissues, with the highest expression in adipose tissue. The enzyme is specific for monoacylglycerol and it does not hydrolyze TG or DG. It seems to be constitutively active since its activity is not affected by hormones or other factors (4, 5, 22).

SKELETAL MUSCLE TRIACYLGLYCEROL HYDROLYSIS

Skeletal myocytes contain a relatively small amount of triacylglycerol (TG). As in adipocytes, they are stored in lipid droplets (LD). The myocyte contain several small LD, 0.3 – 1.5 μm in size. There are two pools of LD in myocytes: subsarcolemmal (smaller) and interfibrillar (larger). LD are mostly found in the vicinity of the mitochondria (9, 23), and their content in type I fibers is two-three times higher than in type II fibers (24). Lipid droplets are movable (25). There is substantial evidence that most FA entering the myocytes are first esterified into TG and moved to LD before oxidation (26-29). This proves the key role of LD in FA supply for oxidation. A combination of strength and endurance training was found to reduce the number of LD and their diameter in the subsarcolemmal region, and not to affect these parameters in the intermyofibrillar region (23). After the discovery of ATGL, its presence was reported in the skeletal muscle of mouse (16), rat (30, 31) and human (32-34). The ATGL mRNA level in the skeletal muscle accounts for approximately 25% of that in the white adipose tissue (35, 36). In humans, ATGL was found only in type I (oxidative) fibers (32). The reduction in ATGL expression was accompanied by TG elevation both in C2C12 myotubes and *in vivo* in the rat tibialis anterior muscle. Overexpression of the enzyme in the cells and in the muscle was accompanied by a reduction in TG content (30). Overexpression of ATGL in cultured C2C12 myotubes increased TG turnover in the cells. It was accompanied by the enhanced activity of peroxisome proliferator-activated receptor δ (PPARδ) and upregulation of some target genes of this receptor. As a result, mitochondrial oxidative capacity increased. Long chain fatty acids are important activators of PPARδ. Therefore, the acids released from endogenous triacylglycerol by overexpressed ATGL are most likely the receptor stimulators. The activation of the receptor would lead to the activation of the enzymes involved in fatty acid oxidation. An involvement of other factors stimulating fatty acid oxidation is also suggested. However, *in vivo* overexpression of ATGL in mouse tibialis anterior muscle did not significantly elevate fatty acid oxidation. It was suggested that *in vivo* ATGL is not a major factor regulating mitochondrial activity in skeletal muscles (27).

Skeletal muscles contain CGI-58 and G0S2 (19, 20). Fasting increases CGI-58 expression in both skeletal and heart muscles (19). Skeletal and heart muscle CGI-58-deficient mice develop TG accumulation in each skeletal muscle type and in the myocardium, respectively. The lack of CGI-58 did not affect the *in vitro* triacylglycerol hydrolytic activities in skeletal muscle homogenates. Also, PPARα and PPARβ/δ-activated gene expression involved in mitochondrial fatty acid oxidation and mitochondrial oxidation of 14C-labeled oleic acid in skeletal muscles remained stable. Addition of recombinant CGI-58 increased triacylglycerol-hydrolytic activity of the muscle homogenates similarly in the control and in coactivator-deprived mice. Interestingly, exercise reduced TG level in tibialis anterior muscle (only this muscle was examined) in the control but not in the CGI-58 knockout mice. The exercise did not affect *in vitro* the triacylglycerol-hydrolytic activity of the muscle homogenates. However, the exercise increased the expression of lipoprotein lipase mRNA and CD36. Lipoprotein lipase is bound to the endothelium and hydrolyses plasma triacylglycerol. Thus, it increases availability of fatty acids for the myocytes. CD36 transports fatty acids across the plasma membrane. Therefore, the supply of plasma fatty acids to the contracting myocytes compensates the lack of endogenous triacylglycerol hydrolysis. Plasma free fatty acids entering the myocytes would maintain the activity of the PPARα and PPARβ/δ and thus of the genes involved in mitochondrial oxidation. It should be added that lack of endogenous triacylglycerol utilization was also compensated by increased glucose utilization in the ATGL knockout group (37). As mentioned above, G0S2 inhibits ATGL in the adipose tissue. Very recently, its role in the skeletal muscle was examined in details. In mice, G0S2 content in the soleus (oxidative muscle, type I muscle) is higher than in the gastrocnemius (a mixture of type IIa and type IIx fibers) and in the extensor digitorum longus muscle (EDL is composed predominantly of type IIx fibers) (38). Recombinant human G0S2 reduced ATGL activity in lysates of both mice soleus and EDL and in human vastus lateralis. It also inhibited the control and CGI-58 stimulated ATGL activity in C0S-7 cell extracts overexpressing human ATGL. Overexpression of G0S2 in human primary myotubes resulted in TG accumulation, reduction in lipolysis and FA oxidation. On the contrary, G0S2 knockdown in the cells activated lipolysis and fat oxidation. Knockdown of G0S2 and ATGL resulted in the elevation in TG content, blunted FA release and oxidation. The results clearly indicate that G0S2 inhibits lipolysis indirectly, inhibiting ATGL activity (38).

EFFECT OF EXERCISE ON MUSCLE ADIPOSE TRIGLYCERIDE LIPASE, COMPARATIVE GENE IDENTIFICATION-58 AND G0/G1 SWITCH PROTEIN 2

It was reported that intramuscular TG are utilized during prolonged exercise both in human and rat muscles. This was later questioned by some researches, who argued that the early data were obtained using biopsy samples of the muscles and thus the samples might have been contaminated with fat cells residing between the myocytes and along the vessels and nerves.

However, histochemical data and results of TG content determination in isolated myocytes clearly confirmed that exercise increases the utilization of muscle TG, mostly in type I fibers (39). However, enzymes responsible for muscle TG hydrolysis had remained unknown until 1999 when Langford *et al*. (40) reported on the presence of hormone sensitive lipase in rat skeletal muscles. The activity of the enzyme in high oxidative muscles was much higher than in glycolytic muscles. Its activity in the incubated soleus was enhanced by adrenaline (40) and transiently by contractile activity (41). The researchers concluded that hormone sensitive lipase (HSL) was the key enzyme responsible for hydrolysis of muscle TG. However, the data obtained in human muscle were controversial. Thirty min exercise of moderate intensity was found to increase HSL activity, which returned to normal after 60 min exercise (42). However, increased activity of the enzyme after 60 min exercise of similar intensity was also reported (43). On the other hand, a dissociation was observed between HSL activity and muscle TG mobilization during 90 and 180 min exercise of moderate activity (44). Finally, Alsted *et al*. (31) provided very strong evidence for the crucial role of ATGL in the activation of muscle TG lipolysis during exercise. Using the incubated soleus muscle they found that: (a) electrical stimulation of the rat muscle produced reduction in the muscle TG content, (b) acute inhibition of HSL (by specific inhibitor of the enzyme) did not inhibit the muscle TG utilization during contractile activity, (c) in the soleus of HSL knock-out mice the contraction-induced reduction in the muscle TG content, LD number and size was similar to that in wild-type mice, (d) ATGL and HSL activity accounted for about 98% of total TG hydrolase activity in the mouse muscle. The final conclusion was that ATGL plays a major role in muscle TG mobilization during exercise. The discrepancy between the data reported by Langford *et al*. (41) and Alstead *et al*. (31) were ascribed (31, 45) to differences in the experimental protocol. Langfort *et al*. (41) measured HSL activity after exercise using muscle lysates. Alstead *et al*. (31) performed measurements on isolated intact muscle fibers of type I and II, thus avoiding possible contamination with interfibrillar adipocytes. In the latter approach, lipid droplets and regulatory adjacent proteins (CG1-58, G0S2 and perilipins) remained intact on the LD surface. This indicates that the maintenance of the whole lipolytic system intact is necessary to evidence the lipolytic role of ATGL in lipolysis activation in the skeletal muscle. It is currently an accepted view that muscle TG hydrolysis is initiated by the action of ATGL. As in the adipose tissue, HSL would hydrolyze DG an MG-lipase would hydrolyze monoacylglycerols.

Up to now the mechanism of ATGL stimulation during contractile activity has not been recognized. In the rat soleus only low basal phosphorylation of ATGL, PLIN3 and PLIN5 (see below) was reported. It remained unchanged either by treatment with adrenaline or by contractile activity (46), thus indicating that phosphorylation of the enzyme does not play any role in its activation in the skeletal muscle during exercise. The contractile activity of the rat soleus muscle markedly enhanced the interaction between ATGL and CGI-58, suggesting a role of CGI-58 in the activation of the enzyme during contractions (47). It should be added, however, that in another study the elevation in the interaction between ATGL and CGI-58 after contractions of the soleus was insignificant (46).

In humans, endurance training increased ATGL protein expression. It did not affect the expression of CGI-58 and HSL (34, 48). In rats, endurance training increased the content of protein ATGL in each muscle type, the increase being the highest in the white gastrocnemius. It did not affect the content of CGI-58 in either type of muscle. The level of G0S2 protein increased in muscles with high oxidative capacity i.e. the soleus and red portion of the gastrocnemius and remained stable in the white portion of the latter. The training but not acute exercise, increased the G0S2 content in the mitochondria of the red gastrocnemius muscle (other muscle types were not examined in this respect). This would suggest that only chronic physical activity is able to shift G0S2 to the mitochondria. No ATGL was found in the mitochondria (49). In humans, the content of G0S2 protein tightly correlates with the content of muscle ATGL, TG and the activity of cytochrome oxidase (a marker of muscle oxidative capacity). Endurance training increased the content of G0S2 protein (38).

Certain hormones interfere with skeletal muscle triacylglycerol metabolism. Triiodothyronine, e.g. reduces the content of triacylglycerol and elevates the content of diacylglycerol in the soleus. It would suggest activation of ATGL by the hormone (50). However, a role of hormones in regulation of ATGL activity in skeletal muscles remains to be elucidated.

SKELETAL MUSCLE PERILIPINS

Skeletal muscle LD proteome contains over 300 proteins (51). The perilipin (PLIN) family is the best investigated family of the protein pool. There are five isoforms of PLIN, named PLIN1, 2, 3, 4 and 5. PLIN1 has 3 isoforms: A, B, C (52, 53). In the adipose tissue, under basal lipolysis, CGI-58 is bound to un-phosphorylated PLIN1 and is inactive (2-4). Lipolysis in the adipose tissue is activated by different factors and catecholamines are the principal ones. As a result of stimulation, protein kinase A (PKA) is activated and phosphorylates PLIN1 and HSL. Phosphorylated PLIN 1 dissociates CGI-58. Released CGI-58 binds to ATGL and activates it (2-4). Phosphorylated (active) HSL translocates to LD and hydrolyses DG (2-4). PLIN1 knockout mice are lean but have normal body weight. The plasma free fatty acid level is normal and triacylglycerol content is somewhat higher that in control animals. They consume the same amount of food and have normal exercise activity (54). The skeletal muscles lack PLIN 1 (55-58), although some authors found very low expression of PLIN1 in the muscles (59, 60). However, it probably originates from adipose cells residing between muscle fibers. The role of PLIN 2-5 in lipolysis regulation in the skeletal and heart muscle will be discussed below.

Lack of PLIN1 in the skeletal muscles raises a question of how ATGL is activated in the muscles, both at rest and during contractile activity. It is hypothesized that PLIN2, PLIN3 or PLIN5 might play a key role of mediators in the process (61). However, strong, direct proofs supporting this hypothesis are still lacking. Several data have been accumulated so far regarding the effect of exercise and training on the behavior of particular perilipins in different muscle types. The mRNA expression of perilipins was reported in both types of human skeletal muscle fibers. The expression of PLIN2-5 in type I fibers was higher than in type II fibers. The expression of PLIN3 was much lower than the expression of PLIN2, PLIN4 and PLIN5 (24, 61-64). The strength training did not affect the expression of either perilipin whereas endurance training doubled the expression of PLIN2 and PLIN3 (60). Most of the PLIN2 present in skeletal myocytes colocalized LD, whereas the remaining part remained in the cytoplasm (62, 64, 65). In PLIN2 knocked out C2C12 cells, LD formation and TG storage were reduced and palmitate incorporation into diacylglycerols and phospholipids was increased. Overexpression of PLIN2 in the cells as well as in the tibialis anterior muscle (*in vivo*) increased TG accumulation and oxidative capacity (66). The contractile activity of the isolated, incubated soleus muscle reduced the interaction between PLIN2

and ATGL, and increased the interaction between ATGL and CGI-58. These changes would facilitate lipolysis (47). Another proof for the involvement of PLIN2 in muscle TG mobilization was obtained in human skeletal muscle. One-hour exercise of moderate intensity reduced the TG content in type I fibers by 50%, which was a consequence of a reduction both in LD intensity and size. TG content in type II fibers remained stable. The exercise did not affect the PLIN2 content in either type of fiber. However, the exercise reduced the number of LD associated with PLIN2 by approximately 30%, whereas the number of PLIN2 null LD in type I fibers remained stable. No such changes occurred in type II fibers. This clearly indicates that LD containing PLIN2 are preferentially utilized during exercise (24). The results indicate that the presence of PLIN2 is necessary to mobilize TG stored in lipid droplets. However, so far the exact mechanism of its action has not been elucidated.

The presence of PLIN5 was found to be restricted to the tissues with high rate of lipolysis, including type I skeletal muscle fibers and cardiac muscle (57, 65), which indicates its involvement in FA oxidation. PLIN5 was present both on lipid droplets as well in the cytosol (57, 65). Contrary to PLIN2, PLIN5 was also found in the mitochondria (64, 67). Deletion of PLIN5 in mice reduced TG content and increased the content of ceramide and sphingomyelin in the soleus. It did not influence FA uptake, oxidation and incorporation into TG in the muscle. Mitochondrial function also remained unchanged. However, oxidation of TG-derived FA increased (68). Overexpression of PLIN5 in the rat anterior tibialis muscle increased TG content and LD size. The content of ATGL protein increased, whereas that of CGI-58 protein remained stable in the muscle. Mitochondria isolated from the muscle did not increase FA oxidation. However, oxidation of 14C-palmitate increased in the muscle homogenates containing PLIN5 coated LD. This would suggest that PLIN5 directs FA from lipid droplets to mitochondria. Overexpression of PLIN5 in the muscle increased the expression of PPARα and peroxisome proliferator-activated receptor gamma co-activator 1α (PGC1α) controlled genes responsible for FA oxidation (67, 69). In the isolated, incubated soleus muscle, PLIN2, PIN3 and PLIN5 were found to interact with ATGL at rest and the interaction was not changed after contractile activity. Only PLIN3 and PLIN5 interacted with CGI-58. In the case of PLIN2, the interaction with ATGL decreased after stimulation. The PLIN proteins were suggested to work together in the regulation of lipolysis (47).

At rest, the number of lipid droplets in the isolated rat soleus was observed to decrease exponentially from sarcolemma to the fiber center. Electrical stimulation of the muscle decreased the content of TG and changed the distribution of lipid droplets from exponential to linear. It did not affect the total content of PLIN2 and PLIN5. The distribution of PLIN2 decreased exponentially from sarcolemma to the fiber center at rest and after stimulation. The distribution of PLIN5 decreased linearly both at rest and during stimulation. Colocalization of PLIN2, PLIN5 and lipid droplets remained stable after stimulation. However, the exact meaning of these observations still remains unclear (70). In human vastus lateralis, 55.4% of PLIN5 was found to colocalize with LD and it did not change during 1 hour exercise. Also, colocalization of PLIN 5 with mitochondria was stable. The content of TG and PLIN5 remained stable after the exercise. Half of ATGL and 58.5% of CGI-58 colocalized with LD at rest and the percentages remained stable during exercise (71). Both sprint interval (63) and endurance (63, 72) training increased TG content and expression of PLIN 2 and PLIN5 in vastus lateralis muscle. Pre-training acute exercise reduced the number of PLIN2 and PLIN5 lipid droplets and it did not affect the number of the PLIN's-null lipid droplets. The same exercise after the two types of training reduced the number of both PLIN2-LD and PLIN2-null LD, thus suggesting utilization of both pools of LD. No PLIN5-null LD was utilized during acute exercise after training. It was concluded that the increased content of both PLIN2 and PLIN5 following either training protocol promoted utilization of the muscle TG during exercise (63). Ageing was found to increase the expression of PLIN2 in human skeletal muscle, whereas the expression of PLIN5 remained unchanged with age. Reduced mobility of hindlimbs in older subjects resulted in the elevation of PLIN2 expression and reduction in PLIN5 expression. In mice, disuse caused by denervation increased the expression of PLIN2 and reduced the expression of PLIN5. Increased expression of PLIN 2 correlated with muscle strength reduction and increased expression of selected factors involved in muscle atrophy. These data indicate a role of PLIN2 in the development of sarcopenia (73). Another study showed that the content of PLIN2-PLIN5 proteins in women was higher than in man and it was similar in obese and lean subjects. Endurance training increased the content of PLIN5 in both sexes. Only PLIN5 correlated with muscle TG volume. This further indicates a role of PLIN5 in the regulation of muscle TG metabolism (72). In one study, researchers thoroughly examined human skeletal muscle PLIN4 to find that PLIN4 mRNA expression in the muscle was higher than mRNA expression of other perilipins. PLIN4 was located mostly at or in close proximity to the sarcolemma. The content of PLIN4 mRNA in type I fibers was higher than in type II fibers. In humans, combined endurance and strength training reduced the expression of PLIN4 and PLIN2 mRNA. There was a strong positive correlation between PLIN4 expression and LD area at the sarcolemmal region, but not at the interfibrillar LD area, at rest. Importantly, PLIN4 mRNA expression correlated positively, both before and after training, with the level of phosphatydylethanolamine and phosphatydylcholine. This suggests that PLIN4 may be involved in phospholipid metabolism in the cells (60).

The data presented above show that: (1) perilipins play an important role in the regulation of lipid metabolism in skeletal muscle and (2) further extensive work is needed to clarify the mechanism of their action.

HEART MUSCLE

The heart is continuously contracting and thus requires continuous, adequate supply with energy substrates. Under normal blood concentration, FA contribute to 60 – 70% of energy supply, the remaining part being covered mostly by glucose (74-76). Only some of the FA entering cardiomyocytes are directed to mitochondria and oxidized. Most are esterified to TG before being oxidized. This indicates a role of intracellular TG in FA metabolism in cardiomyocytes (75, 77), which store TG in LD. The size of LD in cardiomyocytes is around 0.50 μm, being similar to the size of LD in skeletal myocytes (78). The level of ATGL mRNA in the myocardium equals approximately 25% of its level in the white adipose tissue (16). The lipolysis of endogenous TG in the heart was found to be processed by the same enzymes as in other cell types (79). However, certain specific features of cardiac TG lipolysis regulation were revealed (79). Recent studies showed that the heart TG does not only supply cardiomyocytes with FA but also plays a role in the regulation of the heart function (80). ATGL knockout mice were observed to develop severe accumulation of TG in different tissues including the myocardium (81). In the latter, it was a consequence of increased size and number of LD so that the content of TG increased up to 20-fold. Steatosis of the myocardium caused by ATGL deletion resulted in myocardial fibrosis and severe reduction in the ejection fraction. It led to cardiac insufficiency and premature death of animals (81). HSL knockout mice accumulated DG but not TG in the

myocardium, which points out to the role of the enzyme in the heart DG catabolism (21). Unlike ATGL knockout mice, mice lacking ATGL in all tissues, with the exception of the heart, did not accumulate TG in the myocardium and cardiac failure did not develop. The mice of both groups were not able to increase the plasma FA level during exercise. As a result, they utilized more glycogen and developed hypoglycemia during exercise (82). In ATGL-deficient mice, mRNA levels of PPARα/δ target genes, and the expression of PGC-1α and PGC-1β decreased, which resulted in reduced substrate oxidation. This indicates that FA released from endogenous heart TG by ATGL action plays a crucial role in PPARα/δ (83). Cardiac specific ATGL ablation also resulted in myocardial steatosis and hypertrophic remodeling, but it produced only moderate heart contractile dysfunction. Both oxidation and incorporation of oleate into TG were reduced. The expression of mRNA PPARα and PPARα target genes remained stable in fed animals but it was reduced after 12 h fasting. Mitochondrial morphology remained unchanged but their ability to produce ATP decreased. The level of ceramide and long-chain acyl-CoA was stable (84). Further studies provided more data on the role of heart TG in the regulation of the heart function. Heart specific ATGL overexpression increased TG-hydrolase activity in the myocardium nearly 6-fold and reduced the TG content. It also prevented TG accumulation in the heart, caused by fasting. Heart expression of PLIN5 was reduced and the level of DG and ceramide remained stable. Cardiac systolic function was moderately improved. The exercise capacity of the mice increased. The contractile response of isolated cardiomyocytes to isoproterenol increased. FA uptake (oxidation + incorporation into TG) was reduced but glucose usage increased. The mRNA expression of PPARα/δ target genes involved in FA oxidation and mRNA expression of PGC-1α was reduced. Heart-specific ATGL overexpression protected against systolic dysfunction and pathological remodeling of the organ under chronic pressure overload produced by constriction of the aorta in mice (85). In a study on rats, cardiac hypertrophy being a consequence of chronic pressure overload (produced by means of constriction of the abdominal artery) caused a marked reduction in the heart ATGL protein level and TG-hydrolase activity, elevation in TG and ceramide content. Also *in vitro*, phenylephrine (an alpha 1 adrenergic receptor agonist) produced hypertrophy of cardiomyocytes. Overexpression of ATGL in the cells reduced the effect of phenylephrine. The level of ceramide increased in hypertrophic hearts. The increased content of ceramide could be, at least partially, responsible for the induction of cardiac hypertrophy (86). It should be added, however, that the reduction in the content of ATGL in the hypertrophied heart was not confirmed by (87). In the latter study, the protein level of CGI-58 remained stable and the level of G0S2 increased in the hypertrophied hearts. Cardiac specific ATGL overexpression in mice with type 1 diabetes prevented accumulation of TG in the myocardium, lipotoxicity and reduction in the cardiac function (88). Cardiac ATGL overexpression also prevented TG accumulation in cardiomyocytes and development of heart insufficiency in obese mice (89). In the rat, endurance training did not affect the heart ATGL and G0S2 protein levels, but it increased CGI-58 protein level (87). Reports published so far have clearly indicated a crucial role of CGI-58 in ATGL activation and G0S2 in the inhibition ATGL activity in the heart. In the heart and skeletal muscle of CGI-58 knockout mice, ATGL protein content in the myocardium was elevated. In spite of that, they developed cardiac steatosis, hypertrophy and dysfunction. Addition of recombinant CGI-58 to the myocardial lysates restored TG-hydrolytic activity. The expression of PPARα and β/δ dependent genes was impaired, leading to impaired TG metabolism and mitochondrial FA oxidation (37). This indicates a key role of CGI-58 in the activation of ATGL in the heart. A detailed description of G0S2 function in the heart was provided by (90). The expression of cardiac G0S2 mRNA was stronger than its expression in the skeletal muscle. G0S2 expression in the heart was reduced by fasting and elevated over the fed state by subsequent re-feeding. Specific overexpression of G0S2 in the myocardium increased TG content in the heart over 20-fold and caused its enlargement. The TG-hydrolase activity in muscle homogenates was lower than in control mice. This was a consequence of ATGL inhibition by G0S2. The protein levels of ATGL, HSL, MGL and CGI-58 were similar to the values noted in control mice. The results clearly showed that G0S2 is a strong ATGL inhibitor in the heart. Overexpression of G0S2 induced elevation in the heart mass but its contractile ability remained stable. The whole body of G0S2 knock-out mice had unchanged heart structure and function (90). Tachycardia was shown to induce changes in the content of heart diacylglycerol and triacylglycerol (91). However, no data are available on effect of tachycardia on the heart ATGL activity.

HEART PERILIPINS

There are only a few data on the role of perilipins in the heart. PLIN5 knockout mice do not contain LD in cardiomyocytes and they have lower TG level. Therefore, PLIN5 is needed to maintain LD in the cells. Cardiomyocytes isolated from PLIN5-lacking mice take up similar amounts of FA to cardiomyocytes obtained from wild type mice. However, they oxidize more FA at the expense of their incorporation to TG. Lack of PLIN5 was found to increase the production of reactive oxygen species (92). Overexpression of PLIN5 in the heart resulted in an increase in the size and number of LD and severe steatosis. In spite of that, the cardiac function remained only mildly impaired (93, 94). The activity of TG-hydrolase and the content of ATGL and CGI-58 protein were elevated. LD of PLIN5 overexpressed COS-7 cells were partially resistant to the action of ATGL. This would indicate that PLIN5 blocked the access of the enzyme to TG stored in LD (93). PLIN5 overexpression reduced the expression of PPARα, PGC1α and β, and their target genes involved in FA oxidation. In consequence, the function of mitochondria was impaired. This was most likely due to reduced availability of fatty acid ligands that are necessary to activate PPARα (93, 94). However, the mechanism of PLIN5 action on cardiac TG lipolysis remains obscure. PLIN5 may be phosphorylated by protein kinase A (PKA) (46, 68, 95). It has been shown recently that at the basal state, PLIN5 binds both CGI-58 and HSL. Upon stimulation (i.e. by fasting) the cellular level of cAMP increases, which leads to PKA activation. Activated PKA phosphorylates PLIN5 (at serine 155) and HSL. It was hypothesized that phosphorylated PLIN5 dissociates CGI-58. Released CGI-58 bound to ATGL and increased the activity of the enzyme (96).

The role of other PLIN in the regulation of cardiac TG utilization remains unknown. The only exception is a report on PLIN4 action. PLIN4 knockout mice were found to lose TG from the heart (but not from the soleus). Lack of PLIN4 prevented lipid accumulation in the heart during fasting as well protected the heart from steatosis produced by high-fat diet or removal of leptin. The heart function remained unchanged (97).

NEUTRAL LIPID STORAGE DISEASES

Neutral lipid storage diseases (NLSD) is a group of rare genetic disordered featured by the accumulation of triacylglycerol in different tissues, including skeletal and heart muscles (98). A form of NLSD accompanied by myopathy is called NLSD with myopathy (NLSDM). It is caused by

mutations in PNPLA2 gene encoding adipocyte triglyceride lipase (ATGL) (99). Another form of the disease, caused by mutations in the gene, was described by Hirano *et al*. (100, 101). It is manifested by massive accumulation of triacylglycerol in the myocardium and coronary arteries, and is named triglyceride deposit cardiomyovasculopathy (TGCV). Also, a form of NLSD accompanied by ichthyosis, hepatomegaly and mild myopathy was identified and called NLSD with ichthyosis (NLSDI) or Chanarin-Dorfman syndrome. The disorder is caused by mutations in the gene encoding CGI-58, the ATGL activating protein (102, 103). Different clinical symptoms have been associated with the diseases, including skeletal and heart muscle myopathy, liver damage, ataxia, hearing loss, ichthyosis, cataract, nystagmus, strabismus and sometimes mental retardation (104). Regarding the skeletal muscle, the symptoms appear in the third decade and involve both upper and lower limbs, proximal and distal muscle weakness, asymmetrical distribution of muscle weakness, severe deltoid muscle weakness, absence of facial weakness and swallowing difficulties (105). Cardiac myopathy may be so severe that patients may even require heart transplantation (100).

Metabolic studies have provided some important data on the regulation of triacylglycerol metabolism in patients with gene mutations. Cultured fibroblasts obtained from NLSDM patients showed severely reduced triacylglycerol breakdown rate and deficiency in LD-bound neutral lipase activity (99). In the myocardium, samples obtained from TGCV patients showed enormous accumulation of triacylglycerols, and elevation in the expression of mRNA of the peroxisome proliferator-activated receptors α and γ as well mRNA of CD36 and FABP-4 (the transmembrane long-chain fatty acid transporters). Furthermore, fibroblasts obtained from the skin of the patients showed increased uptake of long chain fatty acids and elevation in their incorporation into lipid droplets. Interestingly, in the heart of ATGL knockout mice the expression of the above genes was reduced. A reason for the difference remains unelucidated (101). A pulse-chase study with fluorescent 1-pyrenedecanoic acid on fibroblasts and myoblasts obtained from NLSDM patients showed reduced clearance of the acid as compared to control cells, thus indicating reduced lipolysis rate. Activation of the beta adrenergic receptors with clenbuterol increased the rate of triacylglycerol lipolysis in fibroblasts. This suggests that hormone sensitive lipase remains active in the cells (106). Other PNPLA2 mutations resulted in the production of ATGL able to bind to lipid droplets but having low lipolytic activity (107, 108), which was confirmed by another study (109). The patient examined in the latter study suffered from myopathy but had normal content of neutral fat in the skeletal muscles. The authors concluded that overloading of skeletal myocytes with fat is not the only reason of myopathy. In a patient with NLSDM and TGCV, triacylglycerol accumulated both in the skeletal and heart muscle (110).

The results obtained in humans with NLSD provided clear support for the role of ATGL in the regulation of triacylglycerol lipolysis. They also showed diversity and complexity of the mutations in the genes and their clinical consequences.

SUMMARY AND FUTURE DIRECTIONS

The results obtained over the last 12 years have revolutionized our knowledge on the regulation of lipolysis in different tissues, including skeletal and heart muscle TG. A new enzyme called adipose triglyceride lipase (ATGL) has been discovered. It has been established that ATGL, but not hormone sensitive lipase (HSL), hydrolyzes the first ester bond of the TG moiety and thus initiates the process of TG lipolysis. HSL plays a key role in DG hydrolysis. A crucial role of CGI-58 in the activation and G0S2 in the inhibition of ATGL has been proved. Many data have been collected on the role of perilipins in the regulation of TG metabolism in the muscles. Intracellular TG metabolism has been shown to be strongly related to the cardiac function. The discovery of ATGL, CGI-58, G0S2 and perilipins has opened a new window in our understanding of the process of lipolysis not only in the adipose tissue but also in other cell types. However, the knowledge in the area is still limited. Especially, further studies are needed to better investigate the physiology of perilipins and other proteins present in the lipid droplet coat. It is necessary to recognize whether perilipins (and other lipid droplet proteins) are involved in the pathophysiology of diseases, and research in this direction would also be needed. We still know too little on the regulation of ATGL activity in different cell types. It should be presumed that CGI-58 and G0S2 are not the only proteins involved in the process. However, it is obvious that much more work is required to fully clarify the issue. Certainly, it is a very promising area of research and future results may have important practical implications. Hopefully, they may allow for the development of a new strategy to treat metabolic disturbances of fat metabolism in different tissues.

Abbreviations: ATGL, adipose triglyceride lipase; HSL, hormone sensitive lipase; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylgycerol; LD, lipid droplet; FA, fatty acids; CGI-58, comparative gene identification 58; G0S2, G0/G1 switch protein 2; PLIN, perilipins; PPAR, peroxisome proliferator activated receptor; PGC, peroxisome proliferator activated receptor gamma coactivator; NLSD, neutral lipid storage diseases

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