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ADIPOCYTE LIPOLYSIS

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ABSTRACT

Lipolysis is one of the key functions of the adipose tissue. Dysregulation of lipolysis plays a major role in the manifestation of obesity, diabetes mellitus and coronary heart disease. The last decade has witnessed the elucidation of several mechanisms that explains the process of adipocyte lipolysis and its role in metabolic disorders. With an increase in the number of people suffering from obesity and obesity mediated complications, it is very important to understand key regulatory elements in the mobilization of the adipose tissue. Interaction of various metabolic pathways and players in adipocyte lipolysis offers scope for therapeutic intervention. This paper reviews recent advancements in this field and some noteworthy elements in adipocyte lipolysis have been discussed.

KEYWORDS: **Lipolysis, Obesity, Adipocyte**

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INTRODUCTION

Adipose tissue is the principal fat storage organ in our body and plays a central role in controlling lipid homeostasis. Fatty acids derived from dietary sources which are in excess of the energy requirements are stored in the adipose tissue as triglycerides. Lipolysis is the process of breakdown of stored Triacylglycerol (TAG) in the hydrophobic lipid droplets (LD) by lipases to release glycerol and fatty acids. Adipose tissue in our body is mostly distributed as subcutaneous and visceral depots. Functional dysregulation of visceral adipocytes is a major contributor of metabolic problems as compared to the subcutaneous or peripheral adipocytes. Hypoxia in subcutaneous adipocytes cause adipocytokine deregulation and disrupts lipolytic function[1]. Therefore, most of the problems associated with obesity are majorly contributed by inflammation mediated through lipolysis in the visceral adipocytes. However, when there is an energy deprivation, lipases break down stored triglycerides (TAG) into glycerol and non-esterifiedfatty acids (NEFA)/free fatty acids (FFA) and transports them to liver and skeletal muscle to undergo β- oxidation. The obese show high levels of plasma FFA. The presence of elevated FFA in the blood is due to an imbalance between lipolytic actions of adipocytes and utilization of FFA's by the liver and skeletal muscle. Excess circulating FFA's impairs the skeletal muscle's glucose utilization and thereby promoteshyperglycaemia and glucolipotoxicity across different tissues. Lipolysis activity of adipocytes is highly regulated by the action of hormones like insulin[2] and catecholamine[3,4]. Impairment in glucose homeostasis and whole body insulin resistance promotes lipolysis in the visceral adipocytes and thus contributes to high levels of plasma FFA mobilization to the portal vein.This promotes lipogenesis in liver and skeletal muscles and thus increases insulin resistance[5]. This review considers recent works in the field of adipocyte lipolysis, established and highly accepted signalling mechanisms and key regulatory elements in lipolytic signalling pathways.

KNOWN MECHANISMS AND PLAYERS OF LIPOLYSIS

cAMP DEPENDENT PATHWAYS

Several hormones mediate lipolysis by binding to β- adrenergic receptors expressed on the surface of white and brown adipose tissue[6, 7]. These GPCR's are coupled to G-proteins like Gs/Gi which mediate specific lipolytic response upon activation. For example, Catecholamine binds to the β_2-adrenergic receptor (AR) and activates Gs which activates adenylyl cyclase and increases the cAMP levels. This in turn activates cAMP dependent Protein Kinase A (PKA) and causes Perilipin A and HSL phosphorylation, subsequently breaking down diacylglycerol[8]. G - proteins are highly significant in mediating lipolytic actions of hormones. Therefore, Gs deficiency or decreased Gs activity observed in obese individuals directly correlate with low cAMP levels and reduced lipolysis[9]. Although β_3-AR doesn't have higher expression as compared to other AR's,it can mediate lipolysis when selectively activated by certain bacterial toxins like Pertussis toxins by selective activation of ERK1/2[10, 11]. Gi coupled to β_3-AR is activated uponβ_3-AR activation and restrains cAMP production through adenylyl cyclase but lipolysis is conducted by ERK ½ mediated PKA activation.

cAMP INDEPENDENT PATHWAYS

Apart from the pathways that depend on cAMP levels in adipocytes, lipolysis may occur through pathways that are cAMP independent. This is different from the usual lipolytic route and is less explored.But, there is a clear suggestion that if α1-adrenoreceptors are activated due to stimulation by increased Ca^{2+} level or by the action of agonists then there is an increase in glycerol release owing to lipolysis. It has been speculated that α1-AR and Gq activation leads to Protein Kinase C (PKC)mediated phosphorylation of CREB (cAMP responsive element binding protein) and controls gene expression of the β-AR receptors and ERK1/2 activation[12] thus mediating lipolysis.

CATECHOLAMINE AND INSULIN MEDIATED REGULATION OF LIPOLYSIS

Catecholamine produced by the Chromaffin cells of the adrenal medulla, has an important role in the regulation of lipolysis. Catecholamine binds to β- adrenergic receptors that are coupled to G proteins. This activates adenylate cyclase and produces cAMPwhichactivates PKA and triggers a phosphorylation cascade that is responsible for the phosphorylation of Perilipin[13]. Phosphorylated Perilipin is no more capable of forming the protective layer on the lipid droplets, thus permits HSL activity and aggravates the lipolytic process. Insulin is among the best known anti - lipolytic hormones. The antagonistic nature of insulin is largely due to its capability to lower cAMP levels. Recent findings show that it is primarily due to the negative effects ofinsulin on the expression of Adipocyte Triglyceride Lipase (ATGL).

ROLE OF PKA IN LIPOLYSIS

PKA generally mediates phosphorylation of HSL at Serine residues- 563, 659 and 660[14] and can be monitored *in-vivo* by its translocation from cytosol to the lipid droplet. Previously it was hypothesized that Serine 563 was the only phosphorylation site in HSL. By 2003, other phosphorylation sites of HSL i.e. Serine 659 and 660 were discovered.Further mutagenesis reports confirm the crucial importance of residues 659 and 660 as activation-controlling sites of HSL[15].

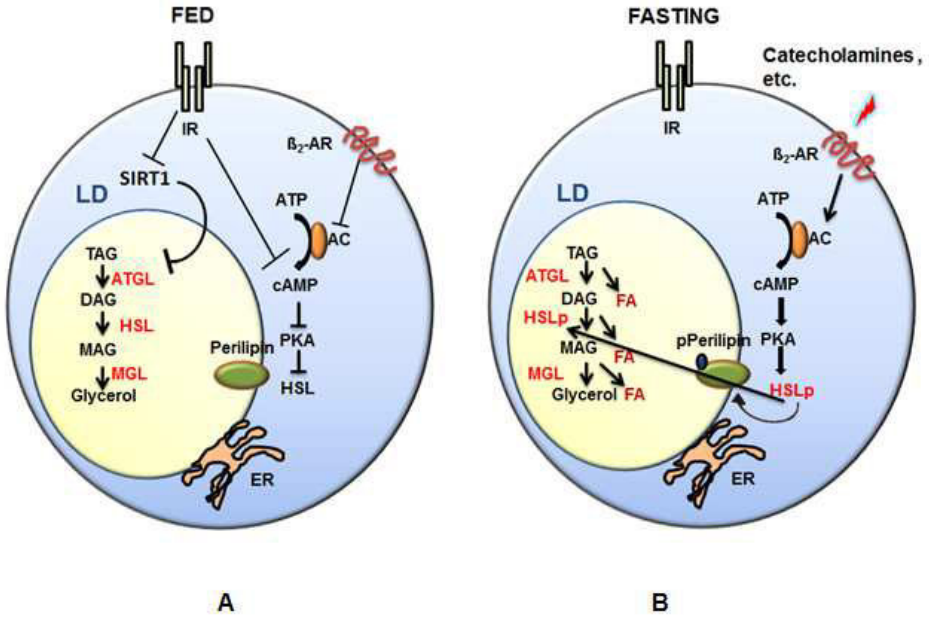


Figure 1 (a) Fed state: Insulin signalling is discontinued attenuating the activation of adenylate cyclase followed by non-phosphorylation of proteins in the lipolytic cascade. **(b)**Fasting state: release of catecholamines, production of cAMP and PKA activation followed by the HSL and ATGL mediated breakdown of stored TAG's.

LIPASES AND LD ASSOCIATED PROTEINS

HSL

HSL is an intracellular lipase which is of 775 amino acid residues in length with a molecular weight of ~ 84kDa[16]. It can cleave Mono, Di and Triacylglycerol moieties, but shows a 10 fold higher preference for Diacylgycerol[17]. The hydrolytic activity of HSL is contributed by PKA mediated phosphorylation at Ser659, Ser660[14, 18] and Ser563[19] thus helpingHSL translocateLipid droplets. This translocation has been hypothesised due to a protein, Lipotrans in that mediates transfer of HSL to the lipid droplet surface. This was identified by using yeast two hybrid screening[20]. HSL interacts with the Adipocyte Lipid Binding Proteins by residues in the N - terminal region. It is considered to be the rate limiting enzyme

of the lipolytic action in adipocytes with lower mRNA expression in the subcutaneous adipose tissue and higher in omental tissue[21]. Apart from the adipose tissue, it is expressed in liver, muscle[22], macrophages, adrenal gland[17] and the testis[23].

ATGL

Adipocyte Triglyceride Lipase has a high expression rate in the adipocytes as compared to other tissues and is responsible for fat mobilization by hydrolysing stored triacylglycerols[24]. A substantial sequence similarity with several proteins from prokaryotes to humans is seen[25, 26] and is responsible for functions from bacterial virulence to nutrient storage[27]. The N-terminal domain of ATGL has a high sequence similarity with Adiponutrin[28] and shares a reciprocal relationship in adipogenesis. siRNA knockdown of ATGL in mouse embryonic fibroblasts decreases both basal and stimulated lipolysis[29]. ATGLwas initially speculated to be localized in the cytosol of adipocytes. Recent reports suggest that ATGL is localizedon the lipid droplets of adipocytes and is present in the cytoplasm for other cells expressing it[30]. Lipid droplet associated proteins play an important role in moderating ATGL function. Perilipin moderates ATGL function in response to β- adrenergic receptor hormonal stimulation and FSP-27 limits its presence on the surface of LD [31]. Insulin inhibits expression of ATGL, playing a major role in the regulation of ATGL via PPARγ[32,33]. However, ATGL expression is also controlled by Insulin viarestrained localization of FoxO1[34] in the cytoplasm.ATGL regulates triglyceride metabolism in intestinal tissues by activation of PPARα following similar steps[35] as its isoforms in other tissues.

PERILIPINS

Perilipin belongs to the PAT (Perilipin, Adipophillin and Tail Interacting protein 47[36,37]) family of proteins that form a protective coating on the LD. It is highly expressed in the white and brown adipose tissue and is the most abundant protein on the LD surface[38,39]. Perilipin has 3 splice variants among which, Perilipin A is the most abundant, both in the white and brown adipose tissue[39]. It acts as a substrate for PKA (Protein Kinase A) mediated phosphorylation owing to its six phosphorylation sites. It has been shown that Ser517 plays a major role in recruitment of ATGL[40] and exposing TAG's for hydrolysis. HSL mediated TAG hydrolysis was initially proposed to be dependent on the phosphorylation state of Perilipin A mediated by PKA[41].Recently, it has been shown that HSL promotes lipolysis by its translocation to LD surfaces in a Perilipin A phosphorylation dependent and independent fashion[42]. The detailed mechanism for this is still unclear.

CGI-58

ATGL as explained above is an adipocyte specific lipase which hydrolyses TAG. It is dependent on CGI-58 (Comparative Gene Identification - 58) for its activity. Structural studies of CGI-58 have shown that its N terminal consists of lipophilic tryptophan rich region which helps it to anchor or localize on the Lipid droplet surface. Deletion of the N-terminal region of CGI-58 resulted in triglyceride accumulation, disrupted its localization potential on the LD surface and affected the enzymatic activity of ATGLin COS-7 cells[43].

TIP47

Tail interacting protein of 47kDa interacts with the cytoplasmic tail of Mannose-6-phosphate receptor and has high sequence similarity with PAT family of proteins. It is found to be localized on the LD surface along with the ADRP's[44]. N-terminal of TIP47is important for its localization and recruitment on LD surface and the C-terminal remains in cytosolic region functioning independently. TIP47 knockdown severely affects the growth and maturation of LD and is thereby considered to be LD protective[45].

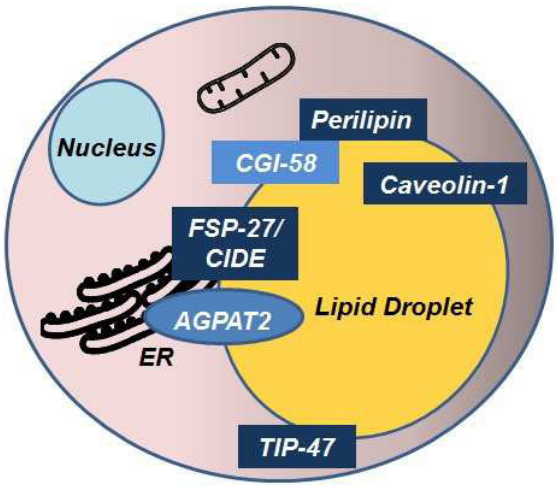
FSP-27

Adipocyte fat specific protein 27 (FSP-27) is highly expressed during adipogenesis[46]. This protein is one of the cell death inducing DNA fragmentation factor 45 (DFF45) like effector protein (CIDE) involved in cellular death[47]. FSP-27 null 3T3-L1 adipocyte cells have shown enhanced basal lipolysis and dispersion of mature LDs into small LD's. FSP-27

localization by GFP tagged FSP-27 constructs showed its presence on the LD surface[48]. Although structurally unrelated to the PAT family of proteins as it doesn't have any PAT specific structural domains, yet this is involved in the formation of large LDs[48] thereby contributes to LD formation and TAG storage[49-51]. However structural information about this protein is yet unavailable and has promisereveal its significance as a LD associated protein.

Lipid Droplet associated proteins

Figure 2



Lipid droplet associated proteins: CGI-58: Comparative Gene Identification-58; FSP-27/CIDE: Fat specific protein-27/cell death inducing DNA fragmentation factor 45 (DFF45) like effector protein; TIP-47: Tail Interacting Protein 47), AGPAT2: Acylglycerol phosphate acyltransferase 2

RECENT MECHANISMS IN ADIPOCYTE LIPOLYSIS

SIRT1-FOX01 MEDIATED LIPOLYSIS

Forkhead BoxProtein (Fox01), a central regulator of metabolic pathways[52]has an implication in adipocyte lipolysis mediated by insulin dependent transcriptional up regulation of ATGL[34].Chakrabarti et al. 2009, have shown that expression of ATGL in adipocyte is regulated by Fox01 but is dependent on the action of Insulin. Insulin inhibits the expression of ATGL[33] by depleting Fox01 nuclear localization and thereby reducing lipolysis or promotes lipolysis if adipocyte insulin signalling is impaired. Recent studies reveal the regulation of Fox01 by SIRT1, one of the seven Sirtuins (SIRT1 through SIRT7) deciphered till date. Sirtuins are NAD^+ dependent proteins which functions by deacetylating histones or transcription factors and regulates their activity in maintaining metabolic stress and related functions[53, 54] in excess nutrient availability[55]. One of the major targets of SIRT1 is Fox01, that is activated by the deacetylation action of SIRT1[56-59]. SIRT1 regulates ATGL expression by deacetylation of Fox01[60] localized in nucleus. This occurs in conditions demanding lipolysis; like prolong fasting or metabolic stress/insulin resistance in adipocytes.

ENDOPLASMIC RETICULUM STRESS MEDIATED LIPOLYSIS

Endoplasmic reticulum (ER) is a membranous organelle which is the major site for the synthesis, folding and trafficking of proteins. Amongst other functions, it is the site for triglyceride droplet formation and maintenance of Ca^{2+} homeostasis. Under stress, folding strategies of ER gets disrupted and gradually there is an aggregation of misfolded proteins, that elicits the adaptive Unfolded Protein Response (UPR)[61-63]. UPR signal transduction is mediated by ER membrane resident proteins; PERK (PKR like eukaryotic initiation factor 2α), IRE-1(Inositol requiring enzyme-1), ATF-6 (activating transcription factor-6)[64]. These three elements controls ER stress by reducing protein translation and increasing ER

chaperones production to mitigate the folding load in the ER lumen. In obesity,there is nutrient excess in the systemic circulation which has profound metabolic consequences in β cells[65, 66], hepatocytes[67] and adipocytes[68, 69], thereby increasing insulin resistance[70] and cellular death[71]in these cells.As ER is the site for triglyceride droplet formation, LD buds off from the ER that is sandwiched between ER membranes. It is evident that there is a close correlation between the lipolytic events which occurs on the TD surface and ER stress. Deng et al. 2011,has reported ER stress associated lipolysisin adipocytes mediated by activation of cAMP/PKA and ERK ½ signalling pathways.They have mentioned that ER stress induction causes simultaneous phosphorylation of ERK ½-JNKpathways and downstream lipolytic events are absolutely dependent on ERK and not JNK[72] which this is not completely explained. Lipolysis could have occurred due to multifactorial events. A probable explanation could be the production of TNF -α by ER stressed adipocytes. TNF-α triggerERK pathway by a cAMP independent mechanism and promote lipolysis[73].The role of individual elements of the UPR and their correlation with lipolytic events has to be further explored to come to a valid conclusion.

mTOR SIGNALING AND LIPOLYSIS

Target of Rapamycin was identified way back in 1991[74]. Several mutational studies in mammalian cells led to cloning and characterization of mammalian target of Rapamycin (mTOR)[75,76]. It plays numerous physiological roles and coordinates cell growth and metabolism by an extensive signalling network. Being a nutrient sensor it effectively senses varying levels of nutrients like amino acids, glucose etc. and is involved in maintaining their homeostasis. Recently its role in lipid metabolism has been explained where it was found to be a promoter of de novo lipogenesis.

MEDIATORS OF LIPOLYSIS

LIPOIC ACID

1,2-dithiolane-3-pentaenoic acid or Lipoic acid is an endogenously secreted compound which is co-factor for several mitochondrial enzymes[77]. Initial studies with Lipoic acid revealed its anti-oxidant properties and its potential role in scavenging reactive oxygen species and rejuvenating endogenous antioxidants like glutathione, Vitamin E and C[78, 79]. Lipoic acid has also been found to increase insulin sensitivity by activating AMPK[80] and induce weight loss in rodent models by increasing energy expenditure[80]. Recent studies reveal that Lipoic acid stimulates lipolysis in adipocyte by phosphorylation of HSL by cAMP mediated activation of PKA and inhibition of Adipocyte – specific Phospholipase A_2 (AdPLA) and Prostaglandin E_2 (PGE_2)[81] which explains its role in reduction of weight in obese models.

LACTATE

Lactic acid is produced as a by-product in metabolic energy expenditure. Lactate level in body is contributed by subcutaneous adipose tissue and muscle. Apparently,obese people have significantly higher lactate levels owing to larger fat mass and chronic intermittent hypoxia.Lactate is more than a metabolic by product and it acts as a signalling molecule being an endogenous ligand of adipocyte specific GRP81[82]. We have conflicting knowledge on the lipolytic action of lactate. Liu and co-workers have shown that lactate binds to GRP81 and internalizes it after activation, thereby supressing lipolysis[82]. On the other hand, recent reports by Hashimoto and co-workers showed that exercise training elevates lactate levels in adipose tissue and skeletal muscle promoting lipolysis by significantly increasing the expression of ATGL, HSL and CGI-58[83].

GLUCOCORTICOIDS

Glucorticoids are steroid hormones having diverse functions in our body and plays a part in promoting lipolysis. It ensures that normal adipose tissue lipolysis mediated by the action of various hormones is maintained by the permissive effect[35, 84]. Studies on isolated rat adipocytes showed that glucocorticoids directly stimulate lipolysis in a dose and time dependent manner by binding to the glucocorticoid receptor present on the adipocytes[85]. Glucocorticoids has a negative effect on the lipolytic action of Tumour necrosis factor α (TNFα) majorly mediated by the action

of ATGL[86]. These presents conflicting view on the effect of glucocorticoid treatment to treat obese individuals. Further work is required to understand the exact effect of hypercortisolemia or glucocorticoid treatment in obese subjects.

DISEASES WITH ALTERED LIPOLYSIS

Obesity is one of the major complications in which there is perturbed lipolytic balance. In most of the observed mutations, ATGL/Desnutrin mutation is notable. ATGL/Desnutrin mutation leads to the deletion of residues leading to truncated C-terminal versions, which causes TAG accumulation in the heart and consequently cardiac arrest. These truncated enzyme versions showed elevated activity but consequently lost their localization potential to the lipid droplets thereby posing a lipolytic defect in the individuals carrying such mutations [87,88]. Apart from this mutations in CGI-58, activator of ATGL have been reported to have high triglyceride accumulation, commonly called as the Chanarin-Dorfman syndrome or Neutral Lipid Storage Disease (NLSD)[89]. At, present we don't have complete structural understanding of the function of ATGL or CGI-58, further investigations will give us crucial information about this LD structural proteins for better management of the diseases.

CONCLUSION AND PERSPECTIVES

Therapeutic application with anti-lipolytic perspectives hypothesizes the use of drugs to prevent lipolysis and thereby decreases serum fatty acid levels. Potent anti-lipolytic drugs like Nicotinamide and its derivatives work on this principle. Although intervention of these drugs might show an initial lowering of the serum fatty acid levels, an eventual backslide to earlier levels results with insulin resistance. In the light of recent advances, the concept of using anti-lipolytic agents for dyslipidaemia needs revision. Its reciprocal may rather be effective by promoting lipolysis and thus increases leanness and heightens fatty acid oxidation[90,91].p53 over expression has been related to endoplasmic reticulum stress due to the triggering of inflammation and NFκB pathways[92]. Activated SIRT1, a histone de-acetylase plays a major role in p53 de-acetylation in cancer cells, whose inhibition causes cell death[93]. To evaluate molecular connections between SIRT1 activation and acetylation or de-acetylation of p53 and its relation to calorie restriction and lipolysis would be interesting. Also, uncovering the mechanisms for lipid droplet formation and the role of different structural proteins in the maintenance of lipid droplets might offer us valuable insights and targets for altering lipid mobilization in the body.

REFERENCES

1. Hosogai N, A Fukuhara, K Oshima, Y Miyata, S Tanaka, K Segawa, S Furukawa, Y Tochino, R Komuro, M Matsuda and I Shimomura, Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation: Diabetes, 56: 901-11, (2007).
2. Carnie J A, D G Smith and M Mavris-Vavayannis, Effects of insulin on lipolysis and lipogenesis in adipocytes from genetically obese (ob/ob) mice: Biochem J, 184: 107-12, (1979).
3. Arner P, Catecholamine-induced lipolysis in obesity: Int J Obes Relat Metab Disord, 23 Suppl 1: 10-3, (1999).
4. Jocken J W and E E Blaak, Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity: Physiol Behav, 94: 219-30, (2008).
5. Arner P, Adrenergic receptor function in fat cells: Am J Clin Nutr, 55: 228S-236S, (1992).
6. Galitzky J, C Carpene, A Bousquet-Mélou, M Berlan and M Lafontan, Differential activation of β1-, β2-and β3-adrenoceptors by catecholamines in white and brown adipocytes: Fundamental & clinical pharmacology, 9: 324-331, (1995).

7. Van Liefde I, A Van Witzenburg and G Vauquelin, Multiple beta adrenergic receptor subclasses mediate the l-isoproterenol-induced lipolytic response in rat adipocytes: Journal of Pharmacology and Experimental Therapeutics, 262: 552-558, (1992).
8. Tansey J T, A M Huml, R Vogt, K E Davis, J M Jones, K A Fraser, D L Brasaemle, A R Kimmel and C Londos, Functional studies on native and mutated forms of perilipins a role in protein kinase A-mediated lipolysis of triacylglycerols in Chinese hamster ovary cells: Journal of Biological Chemistry, 278: 8401-8406, (2003).
9. Carel J C, C Le Stunff, L Condamine, E Mallet, J L Chaussain, P Adnot, M Garabedian and P Bougneres, Resistance to the lipolytic action of epinephrine: a new feature of protein Gs deficiency: J Clin Endocrinol Metab, 84: 4127-31, (1999).
10. Gerhardt C, J Gros, A Strosberg and T Issad, Stimulation of the extracellular signal-regulated kinase 1/2 pathway by human beta-3 adrenergic receptor: new pharmacological profile and mechanism of activation: Molecular pharmacology, 55: 255-262, (1999).
11. Carmen G-Y and S-M Víctor, Signalling mechanisms regulating lipolysis: Cellular signalling, 18: 401-408, (2006).
12. Della Rocca G J, T van Biesen, Y Daaka, D K Luttrell, L M Luttrell and R J Lefkowitz, Ras-dependent Mitogen-activated Protein Kinase Activation by G Protein-coupled Receptors convergence of Gi-and Gq-mediated pathways on Calcium/Calmodulin, Pyk2, and Src kinase: Journal of Biological Chemistry, 272: 19125-19132, (1997).
13. Londos C, D L Brasaemle, C J Schultz, J P Segrest and A R Kimmel, Perilipins, ADRP, and other proteins that associate with intracellular neutral lipid droplets in animal cells: Semin Cell Dev Biol, 10: 51-8, (1999).
14. Anthonsen M W, L Ronnstrand, C Wernstedt, E Degerman and C Holm, Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro: J Biol Chem, 273: 215-21, (1998).
15. Su C L, C Sztalryd, J A Contreras, C Holm, A R Kimmel and C Londos, Mutational analysis of the hormone-sensitive lipase translocation reaction in adipocytes: J Biol Chem, 278: 43615-9, (2003).
16. Laurin N N, S P Wang and G A Mitchell, The hormone-sensitive lipase gene is transcribed from at least five alternative first exons in mouse adipose tissue: Mammalian Genome, 11: 972-978, (2000).
17. Kraemer F B and W-J Shen, Hormone-sensitive lipase control of intracellular tri-(di-) acylglycerol and cholesteryl ester hydrolysis: Journal of lipid research, 43: 1585-1594, (2002).
18. Shen W-J, S Patel, V Natu and F B Kraemer, Mutational analysis of structural features of rat hormone-sensitive lipase: Biochemistry, 37: 8973-8979, (1998).
19. Yeaman S J, G M Smith, C A Jepson, S L Wood and N Emmison, The multifunctional role of hormone-sensitive lipase in lipid metabolism: Advances in enzyme regulation, 34: 355-370, (1994).
20. Syu L-J and A R Saltiel, Lipotransin: a novel docking protein for hormone-sensitive lipase: Molecular cell, 4: 109-115, (1999).
21. Large V, P Arner, S Reynisdottir, J Grober, V Van Harmelen, C Holm and D Langin, Hormone-sensitive lipase expression and activity in relation to lipolysis in human fat cells: Journal of lipid research, 39: 1688-1695, (1998).
22. LANGFORT J, T PLOUG, J IHLEMANN, M SALDO, C HOLM and H GALBO, Expression of hormone-sensitive lipase and its regulation by adrenaline in skeletal muscle: Biochem. J, 340: 459-465, (1999).
23. Stenson Holst L, D Langin, H Mulder, H Laurell, J Grober, A Bergh, H W Mohrenweiser, G Edgren and C Holm, Molecular cloning, genomic organization, and expression of a testicular isoform of

hormone-sensitive lipase: Genomics, 35: 441-447, (1996).
24. Zimmermann R, J G Strauss, G Haemmerle, G Schoiswohl, R Birner-Gruenberger, M Riederer, A Lass, G Neuberger, F Eisenhaber and A Hermetter, Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase: Science, 306: 1383-1386, (2004).
25. Mignery G A, C S Pikaard and W D Park, Molecular characterization of the patatin multigene family of potato: Gene, 62: 27-44, (1988).
26. Banerji S and A Flieger, Patatin-like proteins: a new family of lipolytic enzymes present in bacteria?: Microbiology, 150: 522-525, (2004).
27. Paiva E, R M Lister and W D Park, Induction and accumulation of major tuber proteins of potato in stems and petioles: Plant Physiology, 71: 161-168, (1983).
28. Jenkins C M, D J Mancuso, W Yan, H F Sims, B Gibson and R W Gross, Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities: Journal of Biological Chemistry, 279: 48968-48975, (2004).
29. Okazaki H, J-i Osuga, Y Tamura, N Yahagi, S Tomita, F Shionoiri, Y Iizuka, K Ohashi, K Harada and S Kimura, Lipolysis in the absence of hormone-sensitive lipase evidence for a common mechanism regulating distinct lipases: Diabetes, 51: 3368-3375, (2002).
30. Smirnova E, E B Goldberg, K S Makarova, L Lin, W J Brown and C L Jackson, ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells: EMBO reports, 7: 106-113, (2005).
31. Yang X, B L Heckmann, X Zhang, C M Smas and J Liu, Distinct mechanisms regulate ATGL-mediated adipocyte lipolysis by lipid droplet coat proteins: Molecular Endocrinology, 27: 116-126, (2013).
32. Kershaw E E, M Schupp, H-P Guan, N P Gardner, M A Lazar and J S Flier, PPARγ regulates adipose triglyceride lipase in adipocytes in vitro and in vivo: American Journal of Physiology-Endocrinology And Metabolism, 293: E1736-E1745, (2007).
33. Kershaw E E, J K Hamm, L A Verhagen, O Peroni, M Katic and J S Flier, Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin: Diabetes, 55: 148-57, (2006).
34. Chakrabarti P and K V Kandror, FoxO1 controls insulin-dependent adipose triglyceride lipase (ATGL) expression and lipolysis in adipocytes: J Biol Chem, 284: 13296-300, (2009).
35. Obrowsky S, P G Chandak, J V Patankar, S Povoden, S Schlager, E E Kershaw, J G Bogner-Strauss, G Hoefler, S Levak-Frank and D Kratky, Adipose triglyceride lipase is a TG hydrolase of the small intestine and regulates intestinal PPARα signaling: Journal of lipid research, 54: 425-435, (2013).
36. Brasaemle D L, T Barber, N E Wolins, G Serrero, E J Blanchette-Mackie and C Londos, Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein: J Lipid Res, 38: 2249-63, (1997).
37. Heid H, R Moll, I Schwetlick, H-R Rackwitz and T W Keenan, Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases: Cell and tissue research, 294: 309-321, (1998).
38. Greenberg A S, J J Egan, S A Wek, N B Garty, E J Blanchette-Mackie and C Londos, Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets: J Biol Chem, 266: 11341-6, (1991).
39. Greenberg A S, J J Egan, S A Wek, M C Moos, Jr., C Londos and A R Kimmel, Isolation of cDNAs for perilipins A and B: sequence and expression of lipid droplet-associated proteins of adipocytes: Proc Natl Acad Sci U S A, 90: 12035-9, (1993).
40. Miyoshi H, J W Perfield, 2nd, S C Souza, W J Shen, H H Zhang, Z S Stancheva, F B Kraemer, M S Obin and A S Greenberg, Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated

lipolysis in adipocytes: J Biol Chem, 282: 996-1002, (2007).
41. Brasaemle D L, D M Levin, D C Adler-Wailes and C Londos, The lipolytic stimulation of 3T3-L1 adipocytes promotes the translocation of hormone-sensitive lipase to the surfaces of lipid storage droplets: Biochim Biophys Acta, 1483: 251-62, (2000).
42. Miyoshi H, S C Souza, H-H Zhang, K J Strissel, M A Christoffolete, J Kovsan, A Rudich, F B Kraemer, A C Bianco and M S Obin, Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and-independent mechanisms: Journal of Biological Chemistry, 281: 15837-15844, (2006).
43. Gruber A, I Cornaciu, A Lass, M Schweiger, M Poeschl, C Eder, M Kumari, G Schoiswohl, H Wolinski, S D Kohlwein, R Zechner, R Zimmermann and M Oberer, The N-terminal region of comparative gene identification-58 (CGI-58) is important for lipid droplet binding and activation of adipose triglyceride lipase: J Biol Chem, 285: 12289-98, (2010).
44. Wolins N E, B Rubin and D L Brasaemle, TIP47 associates with lipid droplets: Journal of Biological Chemistry, 276: 5101-5108, (2001).
45. Bulankina A V, A Deggerich, D Wenzel, K Mutenda, J G Wittmann, M G Rudolph, K N Burger and S Höning, TIP47 functions in the biogenesis of lipid droplets: The Journal of cell biology, 185: 641-655, (2009).
46. Danesch U, W Hoeck and G Ringold, Cloning and transcriptional regulation of a novel adipocyte-specific gene, FSP27. CAAT-enhancer-binding protein (C/EBP) and C/EBP-like proteins interact with sequences required for differentiation-dependent expression: Journal of Biological Chemistry, 267: 7185-7193, (1992).
47. Inohara N, T Koseki, S Chen, X Wu and G Núñez, CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor: The EMBO journal, 17: 2526-2533, (1998).
48. Puri V, S Konda, S Ranjit, M Aouadi, A Chawla, A Chakladar and M P Czech, Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage: Journal of Biological Chemistry, 282: 34213-34218, (2007).
49. Nishino N, Y Tamori, S Tateya, T Kawaguchi, T Shibakusa, W Mizunoya, K Inoue, R Kitazawa, S Kitazawa and Y Matsuki, FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets: The Journal of clinical investigation, 118: 2808, (2008).
50. Puri V, J Virbasius, A Guilherme and M Czech, RNAi screens reveal novel metabolic regulators: RIP140, MAP4k4 and the lipid droplet associated fat specific protein (FSP) 27: Acta Physiologica, 192: 103-115, (2008).
51. Toh S Y, J Gong, G Du, J Z Li, S Yang, J Ye, H Yao, Y Zhang, B Xue and Q Li, Up-regulation of mitochondrial activity and acquirement of brown adipose tissue-like property in the white adipose tissue of fsp27 deficient mice: PloS one, 3: e2890, (2008).
52. Accili D and K C Arden, FoxOs at the crossroads of cellular metabolism, differentiation, and transformation: Cell, 117: 421-6, (2004).
53. Sauve A A, C Wolberger, V L Schramm and J D Boeke, The biochemistry of sirtuins: Annu Rev Biochem, 75: 435-65, (2006).
54. Nakagawa T and L Guarente, Sirtuins at a glance: J Cell Sci, 124: 833-8, (2011).
55. Chalkiadaki A and L Guarente, Sirtuins mediate mammalian metabolic responses to nutrient availability: Nat Rev Endocrinol, 8: 287-96, (2012).
56. Daitoku H, M Hatta, H Matsuzaki, S Aratani, T Ohshima, M Miyagishi, T Nakajima and A Fukamizu, Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity: Proc Natl Acad Sci U S A, 101: 10042-7, (2004).
57. Motta M C, N Divecha, M Lemieux, C Kamel, D Chen, W Gu, Y Bultsma, M

McBurney and L Guarente, Mammalian SIRT1 represses forkhead transcription factors: Cell, 116: 551-63, (2004).
58. Nakae J, Y Cao, H Daitoku, A Fukamizu, W Ogawa, Y Yano and Y Hayashi, The LXXLL motif of murine forkhead transcription factor FoxO1 mediates Sirt1-dependent transcriptional activity: J Clin Invest, 116: 2473-83, (2006).
59. Calnan D R and A Brunet, The FoxO code: Oncogene, 27: 2276-88, (2008).
60. Chakrabarti P, T English, S Karki, L Qiang, R Tao, J Kim, Z Luo, S R Farmer and K V Kandror, SIRT1 controls lipolysis in adipocytes via FOXO1-mediated expression of ATGL: J Lipid Res, 52: 1693-701, (2011).
61. Fu S, S M Watkins and G S Hotamisligil, The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling: Cell Metab, 15: 623-34, (2012).
62. Ron D and P Walter, Signal integration in the endoplasmic reticulum unfolded protein response: Nat Rev Mol Cell Biol, 8: 519-29, (2007).
63. Wang S and R J Kaufman, The impact of the unfolded protein response on human disease: J Cell Biol, 197: 857-67, (2012).
64. Kaufman R J, Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls: Genes Dev, 13: 1211-33, (1999).
65. Kharroubi I, L Ladriere, A K Cardozo, Z Dogusan, M Cnop and D L Eizirik, Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress: Endocrinology, 145: 5087-96, (2004).
66. Cunha D A, P Hekerman, L Ladriere, A Bazarra-Castro, F Ortis, M C Wakeham, F Moore, J Rasschaert, A K Cardozo, E Bellomo, L Overbergh, C Mathieu, R Lupi, T Hai, A Herchuelz, P Marchetti, G A Rutter, D L Eizirik and M Cnop, Initiation and execution of lipotoxic ER stress in pancreatic beta-cells: J Cell Sci, 121: 2308-18, (2008).
67. Ozcan U, Q Cao, E Yilmaz, A H Lee, N N Iwakoshi, E Ozdelen, G Tuncman, C Gorgun, L H Glimcher and G S Hotamisligil, Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes: Science, 306: 457-61, (2004).
68. Gregor M F and G S Hotamisligil, Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease: J Lipid Res, 48: 1905-14, (2007).
69. Ohsaki Y, J Cheng, M Suzuki, A Fujita and T Fujimoto, Lipid droplets are arrested in the ER membrane by tight binding of lipidated apolipoprotein B-100: J Cell Sci, 121: 2415-22, (2008).
70. Flamment M, E Hajduch, P Ferre and F Foufelle, New insights into ER stress-induced insulin resistance: Trends Endocrinol Metab, 23: 381-90, (2012).
71. Yin J J, Y B Li, Y Wang, G D Liu, J Wang, X O Zhu and S H Pan, The role of autophagy in endoplasmic reticulum stress-induced pancreatic beta cell death: Autophagy, 8: 158-64, (2012).
72. Deng J, S Liu, L Zou, C Xu, B Geng and G Xu, Lipolysis response to endoplasmic reticulum stress in adipose cells: J Biol Chem, 287: 6240-9, (2012).
73. Souza S C, H J Palmer, Y H Kang, M T Yamamoto, K V Muliro, K E Paulson and A S Greenberg, TNF-alpha induction of lipolysis is mediated through activation of the extracellular signal related kinase pathway in 3T3-L1 adipocytes: J Cell Biochem, 89: 1077-86, (2003).
74. Heitman J, N R Movva and M N Hall, Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast: Science, 253: 905-9, (1991).
75. Fingar D C and J Blenis, Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression: Oncogene, 23: 3151-71, (2004).
76. Loewith R and M N Hall, Target of rapamycin (TOR) in nutrient signaling and growth control: Genetics, 189: 1177-201, (2011).
77. Machado R S, D P Clark and J R Guest, Construction and properties of pyruvate dehydrogenase complexes with up to nine lipoyl domains per lipoate acetyltransferase chain: FEMS

microbiology letters, 100: 243-248, (1992).
78. Scholich H, M E Murphy and H Sies, Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on alpha-tocopherol: Biochim Biophys Acta, 1001: 256-61, (1989).
79. Han D, H J Tritschler and L Packer, Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line: Biochem Biophys Res Commun, 207: 258-64, (1995).
80. Lee W J, K H Song, E H Koh, J C Won, H S Kim, H S Park, M S Kim, S W Kim, K U Lee and J Y Park, Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle: Biochem Biophys Res Commun, 332: 885-91, (2005).
81. Fernandez-Galilea M, P Perez-Matute, P L Prieto-Hontoria, J A Martinez and M J Moreno-Aliaga, Effects of lipoic acid on lipolysis in 3T3-L1 adipocytes: J Lipid Res, 53: 2296-306, (2012).
82. Liu C, J Wu, J Zhu, C Kuei, J Yu, J Shelton, S W Sutton, X Li, S J Yun and T Mirzadegan, Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81: Science Signaling, 284: 2811, (2009).
83. Hashimoto T, K Sato and M Iemitsu, Exercise-inducible factors to activate lipolysis in adipocytes: J Appl Physiol, (2013).
84. Djurhuus C B, C H Gravholt, S Nielsen, S B Pedersen, N Moller and O Schmitz, Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans: Am J Physiol Endocrinol Metab, 286: E488-94, (2004).
85. Xu C, J He, H Jiang, L Zu, W Zhai, S Pu and G Xu, Direct effect of glucocorticoids on lipolysis in adipocytes: Molecular Endocrinology, 23: 1161-1170, (2009).
86. Lee M-J and S K Fried, Glucocorticoids antagonize tumor necrosis factor-α-stimulated lipolysis and resistance to the antilipolytic effect of insulin in human adipocytes: American Journal of Physiology-Endocrinology And Metabolism, 303: E1126-E1133, (2012).
87. Schweiger M, A Lass, R Zimmermann, T O Eichmann and R Zechner, Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5: Am J Physiol Endocrinol Metab, 297: E289-96, (2009).
88. Duncan R E, Y Wang, M Ahmadian, J Lu, E Sarkadi-Nagy and H S Sul, Characterization of desnutrin functional domains: critical residues for triacylglycerol hydrolysis in cultured cells: J Lipid Res, 51: 309-17, (2010).
89. Schweiger M, A Lass, R Zimmermann, T O Eichmann and R Zechner, Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5: American Journal of Physiology-Endocrinology And Metabolism, 297: E289-E296, (2009).
90. del Pozo C H, R M Calvo, G Vesperinas-Garcia, J Gomez-Ambrosi, G Fruhbeck, M A Rubio and M J Obregon, Expression profile in omental and subcutaneous adipose tissue from lean and obese subjects. Repression of lipolytic and lipogenic genes: Obes Surg, 21: 633-43, (2011).
91. Ahmadian M, R E Duncan, K Jaworski, E Sarkadi-Nagy and H S Sul, Triacylglycerol metabolism in adipose tissue: Future Lipidol, 2: 229-237, (2007).
92. Lin W C, Y C Chuang, Y S Chang, M D Lai, Y N Teng, I J Su, C C Wang, K H Lee and J H Hung, Endoplasmic reticulum stress stimulates p53 expression through NF-kappaB activation: PLoS One, 7: e39120, (2012).
93. Jang S Y, S Y Kim and Y-S Bae, p53 deacetylation by SIRT1 decreases during protein kinase CKII downregulation-mediated cellular senescence: FEBS letters, 585: 3360-3366, (2011).