What Is Lipotoxicity?

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Abstract

Enlarged fat cells in obese adipose tissue diminish capacity to store fat and are resistant to the anti-lipolytic effect of insulin. Insulin resistance (IR)-associated S-nitrosylation of insulin-signaling proteins increases in obesity. In accordance with the inhibition of insulin-mediated anti-lipolytic action, plasma free fatty acid (FFA) levels increase. Additionally, endoplasmic reticulum stress stimuli induce lipolysis by activating cyclic adenosine monophosphate/Protein kinase A (cAMP/PKA) and extracellular signal-regulated kinase ½ (ERK1/2) signaling in adipocytes. Failure of packaging of excess lipid into lipid droplets causes chronic elevation of circulating fatty acids, which can reach to toxic levels within non-adipose tissues. Deleterious effects of lipid accumulation in non-adipose tissues are known as lipotoxicity. In fact, triglycerides may also serve a storage function for long-chain non-esterified fatty acids and their products such as ceramides and diacylglycerols (DAGs). Thus, excess DAG, ceramide and saturated fatty acids in obesity can induce chronic inflammation and have harmful effect on multiple organs and systems. In this context, chronic adipose tissue inflammation, mitochondrial dysfunction and IR have been discussed within the scope of lipotoxicity.

Keywords

Obesity • Lipotoxicity • Lipolysis • Free fatty acid (FFA) • Fatty acyl-coenzyme A (FA-CoA) • Diacylglycerol (DAG) • Ceramide • Perilipin • Triglyceride • Fatty acid translocase (FAT)/CD36 • Long-chain fatty acid (LCFA) • Plasma membrane-associated fatty acid binding protein (FABPpm) • Triacylglycerol • Insulin resistance (IR) • Mitochondrial dysfunction • Lipid droplets • Reactive oxygen species (ROS)

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1 Introduction

Obesity is not only associated with lipid accumulation in adipose tissue, but also in non-adipose tissues. Deleterious effects of lipid accumulation in non-adipose tissues are known as lipotoxicity (van Herpen and Schrauwen-Hinderling 2008). In addition to reducing fatty acid clearance from circulation, enlarged adipose tissue mass releases more free fatty acid (FFA) to circulation increase the eventual plasma FFA levels in obesity. Moreover, once plasma FFA levels are elevated, anti-lipolytic action of insulin is inhibited. In this manner the rate of FFA release into the circulation will further increase (Boden 2008; Jensen et al. 1989). The normal plasma FFA concentration is in the range of 350–500 μM. However, the majority of plasma FFAs is bound to albumin. In human skeletal muscle, value of FFA is between 1–10 nmol/g wet muscle tissues. Whole body insulin sensitivity displays a strong negative correlation with muscle fatty acyl-coenzyme A (FA-CoA) content. Exceeding the amount of FA-CoA over 2 μM is associated with a marked reduction in whole body insulin sensitivity (An et al. 2004). Elevated muscle FA-CoA interferes with mitochondrial adenosine triphosphate (ATP) synthesis by inhibiting the electron transport chain and decreasing the inner mitochondrial membrane potential. In this case reduction of FA-CoA oxidation leads to a rise in muscle FA-CoA concentration and exacerbates the mitochondrial dysfunction (Abdul-Ghani et al. 2008). Gradual increases in saturated FFAs diminish insulin-induced glycogen synthesis, glucose oxidation and lactate production. In this case decreases in both mitochondrial hyperpolarization and ATP generation impair mitochondrial functions (Hirabara et al. 2010). Although circulating fatty acids are important for the physiological regulation of a number of processes, high levels of fatty acids are deleterious (Unger 2002). Obesity causes a chronic elevation of circulating fatty acids that can reach to toxic levels within non-adipose tissues. Fatty acids may enter deleterious pathways subsequent to over-accumulation of lipids in non-adipose tissues during overnutrition (Unger 2002). When saturated long-chain fatty acids are in excess, metabolic flux favors synthesis of complex lipids like ceramides and cholesterol esters. Accumulation of these substances results in adverse effects of lipotoxicity such as endoplasmic reticulum stress, inflammation, and insulin resistance (IR) (Summers 2006; van Herpen and Schrauwen-Hinderling 2008). Atherosclerosis, cardiomyopathy retinopathy, nephropathy, neuropathy and endothelial dysfunction are considered as consequences of the elevated circulating fatty acids that reach to toxic levels (Symons and Abel 2013). In this chapter, common mechanisms of obesity-related lipid accumulation in non-adipose tissues have been discussed within the scope of lipotoxicity (Ye 2013).

2 Lipolysis

Obesity is characterized by excessive adipose tissue deposition and increased FFA release that exceeds metabolic demands (Koutsari and Jensen 2006). When the excess lipids are driven into alternative non-oxidative pathways, the storage capacity of adipose tissue has been overcome. A lipid "spill over" may occur from adipose to non-adipose tissues due to over-accumulation of unoxidized long-chain fatty acids (Kusminski et al. 2009). Actually adipose lipolysis is an important process that controls circulating FFA concentrations. Triacylglycerol hydrolysis in adipocytes produces glycerol and FFAs (Londos et al. 1999a). According to the barrier/translocation hypothesis, perilipin family protein constitutes a physical barrier to hormone-sensitive lipase (HSL). Upon perilipin phosphorylation and downregulation, lipid surface accessibility for HSL is enhanced (Londos et al. 1999b). In adipocytes, this is achieved by sequential action of adipose triglyceride lipase (ATGL), HSL, and monoglyceride lipase (Nielsen et al. 2014). Lipolysis is stimulated by various hormones and effectors. In this event, the lipid droplet-associating proteins or perilipin family proteins are polyphosphorylated by protein kinase A (PKA) and phosphorylation is necessary for translocation of HSL to the lipid droplet and enhanced lipoly-

sis (Tansey et al. 2004). In fact PKA-dependent perilipin phosphorylation facilitates perilipin interaction with lipid droplet-associated HSL (Miyoshi et al. 2006). Dexamethasone induces phosphorylation and down-regulation of perilipin that modulates lipolysis. Additionally dexamethasone up-regulates mRNA and protein levels of HSL and adipose triglyceride lipase; these effects are in parallel to increased lipolysis (Xu et al. 2009). A decreased catecholamine response affects alpha- and beta-adrenoceptor sensitivity in adipose tissue, reducing lipolysis and increasing fat stores in obesity (Zouhal et al. 2013). Thus catabolic hormone, adrenaline translocates both phospholipase C-related catalytically inactive protein (PRIP) and its binding partner protein phosphatase 1 and protein phosphatase 2A (PP2A) from the cytosol to lipid droplets. PRIP promotes the translocation of phosphatases to lipid droplets to trigger the dephosphorylation of HSL and perilipin, thus reducing PKA-mediated lipolysis (Okumura et al. 2014). Neuropeptide Y (NPY) promotes proliferation of adipocyte precursor cells and contributes to the pathogenesis of obesity. Although NPY have no effect on basal lipolysis, it potentiates a beta-adrenergic receptor agonist, isoproterenol stimulated lipolysis (Li et al. 2012). TNF-alpha increases lipolysis in differentiated human adipocytes by activation of mitogen-activated protein kinase kinase (also known as MEK, MAPKK), extracellular signal-related kinase (ERK), and elevation of intracellular cyclic adenosine monophosphate (cAMP) approximately 1.7-fold. TNF-alpha induces perilipin hyperphosphorylation by activating PKA (Zhang et al. 2002). Furthermore, TNF-alpha activates the three mammalian mitogen-activated protein kinase (MAPK), p44/42, c-Jun NH2-terminal kinase (JNK), and p38 but only p44/42 and JNK involve in the regulation of lipolysis (Ryden et al. 2002). As mentioned above, cAMP/PKA along with extracellular signal-regulated kinase-1/2 (ERK1/2) is the major early lipolytic signal. Endoplasmic reticulum stress stimuli induce lipolysis by activating cAMP/PKA and ERK1/2 signaling in adipocytes. This lipolytic activation is probably an adaptive response that regulates energy homeostasis. Because of the persistent acceleration of FFA efflux from adipocytes to the bloodstream and various tissues, endoplasmic reticulum stress impairs insulin sensitivity by contributing to lipotoxicity (Deng et al. 2012). Triacylglycerol-rich lipid droplets of adipocytes provide a major energy storage depot for the body. These lipid droplets are coated with one or more of five members of the perilipin family of proteins: adipophilin, perilipin-3 (formerly called TIP47), OXPAT/myocardial lipid droplet protein (MLDP), S3–12, and perilipin. They prevent triglyceride hydrolysis by lipases. Perilipin is the most abundant protein on the surfaces of lipid droplets, and the major substrate for cAMP-dependent protein kinase. In times of energy deficit, perilipin is phosphorylated by PKA and facilitates maximal lipolysis by HSL and adipose triglyceride lipase (Brasaemle 2007). Endoplasmic reticulum stress did not alter the level of perilipin proteins but increased the phosphorylation (Deng et al. 2012). The formation and elevation of reactive lipid moieties and FFA in non-adipose tissues promote cellular dysfunction (lipotoxicity) and programmed cell-death (lipoapoptosis) (Kusminski et al. 2009) (Fig. 8.1).

3 Fatty acid Transport

Cellular long-chain fatty acid (LCFA) uptake involves both passive diffusion and protein-mediated transport which includes fatty acid translocase (FAT)/CD36, plasma membrane-associated fatty acid binding protein (FABPpm), and fatty acid transport protein (FATP). Furthermore, it appears that FAT/CD36, as a key fatty acid transporter, regulates the LCFA utilization in heart and skeletal muscle both under normal conditions as well as in obesity and IR (Koonen et al. 2005). Increased cytosolic FAT/CD36 expression also results in a concomitant increase in mitochondrial FAT/CD36 content in muscle cells. Thus translocation of FAT/CD36 to the mitochondria enhances the mitochondrial fatty acid oxidation capacity. This means that FAT/CD36 influences both LCFA transport across the plasma membrane and also LCFA

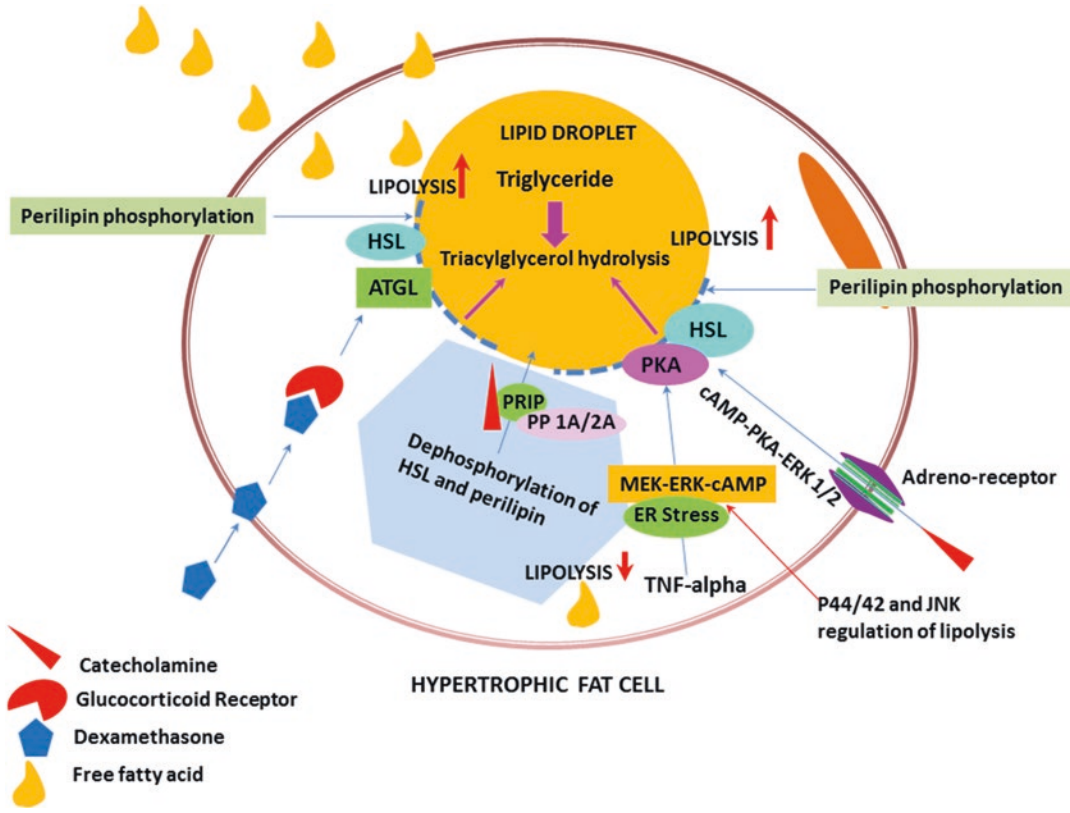

Fig. 8.1 Schematic description of basic steps of lipolysis. Lipolysis is stimulated by various hormones and effectors. The lipid droplet-associating perilipin family proteins are polyphosphorylated by protein kinase A. Phosphorylation is necessary for translocation of hormone-sensitive lipase to the lipid droplet and enhances lipolysis (*HSL* Hormone-sensitive lipase; *ATGL* Adipose triglyceride lipase; *PKA* Protein kinase A; *PRIP* Phospholipase C-related catalytically inactive protein; *PP1A/2A* Protein phosphatase 1A and protein phosphatase 2A; *JNK* c-Jun N-terminal kinase; *MEK* Mitogen-activated protein kinase kinase; *ERK ½* Extracellular signal-related kinase; *cAMP* 3',5'-cyclic adenosine monophosphate; *TNF-alpha* Tumor necrosis factor-alpha; *ER* Endoplasmic reticulum)

transport into mitochondria (Campbell et al. 2004). When saturated and unsaturated LCFAs are presented to the mitochondria for beta-oxidation and to the endoplasmic reticulum for lipid synthesis, only LCFA are used in the synthesis of ceramide. Increase in this metabolite causes lipotoxicity (Brookheart et al. 2009; Listenberger et al. 2001). Another putative fatty acid transporter, FABPpm overexpression increases the rates of LCFA transport across the sarcolemma. This effect is independent of any changes in FAT/CD36. Nevertheless, the overexpression of FABPpm alone is not sufficient to induce a parallel increment in palmitate transport and metabolism (Clarke et al. 2004). While FABPpm contributes to increasing sarcolemmal LCFA transport, it can not participate in the LCFA transport into the mitochondria (Holloway et al. 2007). On the other hand, insulin regulates protein expression of FAT/CD36, but not FABPpm, via the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) insulin-signaling pathway. Eventually the insulin-induced increase in FAT/CD36 protein in a time- and dose-dependent manner results in an increased rate of LCFA transport. Meanwhile, an increase in plasmalemmal FAT/CD36 occurs (Chabowski et al. 2004). However, high levels of saturated FFA-

induced mitochondrial dysfunction are associated with disruption of PI3K/Akt insulin-signaling pathway (Hirabara et al. 2010). Both FAT and FABPpm are present on the muscle membrane and are important in regulating the uptake of fatty acids into skeletal muscle. In fact, the LCFA transport proteins are also located in several subcellular domains (Bonen et al. 2000). The peroxisome proliferator-activated receptor-gamma (PPAR-gamma) regulates the adipogenesis and insulin responsiveness. FABP4 triggers the proteasomal degradation of PPAR-gamma. Therefore, higher FABP4 in human visceral fat is an important factor in the development of obesity-related morbidities (Garin-Shkolnik et al. 2014). High saturated fatty acids and trans fatty acids intake favors a proinflammatory status that contributes to development of IR. While excess fatty acids induce hepatic IR, they also impair insulin clearance in obese non-diabetic humans (Carpentier et al. 2000). Therefore, IR and oxidative stress play an important role in development and progression of nonalcoholic fatty liver disease (Angulo 2007). FATPs are a family of six integral membrane proteins with an extracellular/luminal N-terminal and C-terminal domain with fatty acyl-CoA synthetase activity and therefore FATP proteins have the ability to trap fatty acids intracellularly (Watkins 2008).

Fatty acids may exert their effects directly by binding to cell surface receptors or to intracellular transcription factors. Monounsaturated fatty acids are more potent PPAR ligands than saturated fatty acids. Monounsaturated fatty acids activate the nuclear transcription factors PPAR-alpha and PPAR-gamma which respectively promote lipid detoxification via fatty acid oxidation and safe fatty acid storage into triglycerides (Nolan and Larter 2009). Channeling of non-esterified free fatty acids (NEFA) towards storage in the form of neutral lipids in lipid droplets protects the cell against palmitate-induced endoplasmic reticulum stress (Bosma et al. 2014). PPAR-gamma senses incoming non-esterified LCFAs and induces the pathways to store LCFAs as triglycerides (Nakamura et al. 2014). Triglycerides serve a storage function for long-chain NEFA and their products such as ceramides and DAGs. Their toxicity is originated from failure of esterification or breakdown of the triglycerides (Listenberger et al. 2003). PPAR-gamma expression is increased in humans with metabolic syndrome. Enhanced expression of several PPAR-regulated genes mediates fatty acid uptake/oxidation and triacylglycerol synthesis. Fatty acid oxidation and triacylglycerol droplet size are increased (Son et al. 2010).

Obesity-related reduction in skeletal muscle fatty acid oxidation is attributable to the reduced mitochondrial content of fatty acids, not to mitochondrial dysfunction. Thus, the mitochondrial FAT/CD36 content of lean muscle is not different than that of the obese muscle. Eventually FAT/CD36 significantly predicts the ability of mitochondria to oxidize fatty acids, independent of body mass index (BMI) status (Holloway et al. 2009). However, trafficking of fatty acid transporters between the intracellular compartments and the plasma membrane is altered in insulin-resistant skeletal muscle. In obesity FAT/CD36 permanently relocates to plasma membrane, hereby contributes to IR by increasing influx of fatty acids into muscle cells (Chabowski et al. 2007). Insulin-resistant individuals have a reduced rate of fat oxidation compared with insulin-sensitive individuals. Decreased mitochondrial fat oxidative capacity leads to an increase in intracellular fat content (Kelley and Simoneau 1994).

In physiological conditions, non-adipose tissues contain very few triglycerides. Ectopic lipid accumulation may occur in the setting of high concentration of serum triglycerides and long-chain NEFAs which are also referred to as FFAs. Reasonably, ectopic fatty acid accumulation results from disequilibrium between FFAs uptake from the environment and consumption through the mitochondrial oxidation. In this situation, the more specific disturbance due to lipid overload in non-adipose tissues is DAG and/or Acyl-CoA-related interference with insulin signaling appear. (Schaffer 2003; van Herpen and Schrauwen-Hinderling 2008). Actually intracellular utilization of LCFAs is subdivided into three steps; initial uptake across the plasma membrane, activation by esterification with coenzyme A, and

subsequent metabolism. Long chain acyl-CoA synthetases (ACSLs) not only activate fatty acids for intracellular metabolism but are also involved in the regulation of uptake. Multiple different long chain ACSLs are expressed simultaneously in the same cell type but differ in their subcellular localization. ACSLs localize to either the endoplasmic reticulum or to mitochondria and can regulate the extent of fatty acid uptake (Digel et al. 2009).

Palmitic acid mainly occurs as its ester in triglycerides and it is most common fatty acids found in meats, cheeses, butter, and dairy products. According to the World Health Organization, evidence is convincing that consumption of palmitic acid increases risk of developing cardiovascular diseases, placing it in the same evidence category as trans fatty acids ("WHO_TRS_916.pdf" n.d.). Palmitate-induced inhibition of carnitine palmitoyltransferase I, subsequent accumulation of ceramide, and inhibition of electron transport complex III cause cytochrome c release and apoptosis (Sparagna et al. 2001). The most important biological function of L-carnitine is to transport fatty acids into the mitochondria. However, carnitine insufficiency impairs entry of fatty acids into the mitochondria and consequently disturbs lipid oxidation (Reuter and Evans 2012). It is proposed that carnitine transport system at the contact sites between the outer and inner mitochondrial membranes comprises the long-chain acyl-CoA synthase and porin components. The mitochondrial carnitine system is also necessary in beta-oxidation of LCFAs. The malonyl-CoA sensitive carnitine palmitoyltransferase I/malonyl-CoA system regulates fatty acid oxidation depending on the tissue's energy demand (Kerner and Hoppel 2000). Furthermore, Listenberger et al. proposed that saturated fatty acid, palmitate-induced apoptosis occurs through the generation of reactive oxygen species (ROS) (Listenberger et al. 2001). Thus, palmitate overload rapidly increases the saturation of phosphatidylcholine (PC) and triacylglycerol in endoplasmic reticulum membranes. Markedly dilated rough endoplasmic reticulum due to increased accumulation of PC and TAG are associated with oxidative stress. These alterations initiate the flux of calcium from the endoplasmic reticulum stores to the mitochondria. The loss of mitochondrial membrane potential ultimately leads to palmitate-induced cell death (Borradaile et al. 2006). During over-nutrition, higher plasma leptin concentration stimulates phosphorylation of signal transducer and activator of transcription 3 (STAT3) in Janus kinase (JAK)/STAT pathway. Subsequently phosphorylated STAT3 enters the nucleus and regulates transcriptional activity of its target genes including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha), carnitine palmitoyltransferase I (CPT-1) and acyl-CoA oxidase (ACO). While PGC-1alpha involves in mitochondrial biogenesis, CPT-1 and ACO are responsible for the fatty acid oxidation. However, STAT3 reduces the expression of lipogenic enzymes; acetyl coenzyme A (CoA) carboxylase (ACC) and fatty acid synthase (FAS) (Unger 2003a). The adenosine monophosphate (AMP)-activated protein kinase (AMPK) is stimulated by ATP depletion and it blocks energy consuming processes. Moreover, higher plasma leptin selectively stimulates phosphorylation and activation of the alpha2 catalytic subunit of AMPK. Simultaneously leptin suppresses the activity of ACC, thereby stimulates the oxidation of fatty acid (Minokoshi et al. 2002). Obesity is associated with hypoadiponectinemia. Adiponectin regulates fatty acid utilization via AMPK-dependent mechanisms that enhance mitochondrial fatty acylCoA import (generation of malonyl-CoA by ACC). Through induction of CD36 translocation, fatty acid uptake increases. Eventually, adiponectin accelerates complete oxidation of fatty acids. Thereupon, accumulation of toxic lipid intermediates is prevented in a large scale (Fang et al. 2010).

ACC synthesizes malonyl-CoA (MCA) which is an inhibitor of fatty acid oxidation in mitochondria. Phosphorylation or inactivation of ACC controls MCA activity (Winder and Hardie 1996). MCA is a powerful inhibitor of CPT-1-mediated fatty acid oxidation (McGarry 2002). Lack of the leptin effect increases the ACC activity and produces more MCA. Virtually more fatty acid and triglycerides are synthesized however, they are less oxidized (Unger 2003a). Leptin

infusion markedly decreases the expression of key enzymes of the de novo fatty acid synthesis such as ACC, fatty acid synthase, and stearoyl-CoA desaturase-1. In this case, leptin treatment regulates adipose triglyceride lipase/hormone sensitive lipase/diacylglycerol (DAG) transferase 1 expression and alters fatty acid-triacylglyceride cycling in adipose tissue. Consequently, lipolysis and fatty acid oxidation are increased (Gallardo et al. 2007). In obesity-prone animals, carnitine palmitoyltransferase 1B expression is lower in intra-abdominal fat, however stearoyl-CoA desaturase 1 expression is higher in subcutaneous fat. Increased fat accumulation in obesity may be caused by impaired oxidative capacity due to decreased carnitine palmitoyltransferase 1B levels in the white adipose tissue (Ratner et al. 2015). Overexpression of human carnitine palmitoyltransferase-1A significantly reduces the content of intracellular NEFAs and attenuates fatty acid-evoked IR and inflammation via suppression of JNK. These changes in enzyme levels are accompanied by an increase in fatty acid uptake and a decrease in fatty acid release (Gao et al. 2011). However Schenk and Horowitz suggested that the increased rate of whole body fatty acid oxidation is correlated with an increase in the CD36-associated carnitine palmitoyltransferase-1, but not with carnitine palmitoyltransferase-1 alone (Schenk and Horowitz 2006).

4 Lipotoxicity and Mitochondrial Dysfunction

The fatty acid-overload effects mitochondria in a three-step fashion. At first, the production of ROS occurs in the early phase of fatty acid accumulation. In the second step, increase in mitochondrial proton conductance or the uncoupling of the oxidative phosphorylation is evident. At the last step, fatty acids can provoke the permeabilization of the outer mitochondrial membrane (Listenberger and Schaffer 2002; Rial et al. 2010; Schönfeld and Wojtczak 2008). Once mitochondrial membrane permeabilization (MMP) has been induced, the release of catabolic hydrolases and activators from mitochondria is facilitated. These catabolic enzymes as well as the cessation of the bioenergetic and redox functions of mitochondria finally lead to cell death (Crompton 1999; Kroemer et al. 2007). Really, fatty acids cause oxidative stress and alterations in mitochondrial structure and function. Thus, the uncoupling of the oxidative phosphorylation is one of the most recognized deleterious effects of fatty acid. The fatty acid interaction with the carriers leads to membrane depolarization and/or the conversion of the carrier into a pore (Rial et al. 2010).

Long-chain saturated FFAs induce apoptosis in a dose-dependent manner. Both saturated and unsaturated exogenous long-chain FFAs are directed to the mitochondria for beta-oxidation and to the endoplasmic reticulum for complex lipid synthesis. However only long-chain saturated fatty acyl CoAs serve as substrates for de novo ceramide synthesis. Ceramide is a lipid second messenger involved in initiation of apoptosis (Brookheart et al. 2009). Excessive amount of lipid metabolites like DAG, ceramide and saturated fatty acids in obesity can induce chronic inflammation and have harmful effect on multiple organs and systems through driving these metabolites into alternative non-oxidative pathway. FFAs and their metabolites take part in the structure of membrane and intracellular signaling or ATP generation. However excessive fat accumulation in non-adipose tissues, including the pancreas, heart, liver, kidney and blood vessel wall results in mitochondrial dysfunction (Brookheart et al. 2009).

Fatty acids that are taken up into heart and skeletal muscle are primarily oxidized or stored as triacylglycerols. As long as the fatty acid uptake and metabolism remains appropriately balanced, metabolic dysregulation does not occur. If there is a defect in mitochondrial oxidative phosphorylation, IR is associated with an increase in intramyocellular lipid content. This increase is attributed to the mitochondrial dysfunction (Möhlig et al. 2004). In this case, secondary to diminished fat oxidation, IR develops. The high rates of fatty acid catabolism in insulin-resistant muscles are attributed to incomplete fat oxidation, in which a large proportion of fatty

acids enter into the mitochondria but are only partially degraded. Unchanged rates of fatty acid oxidation can be accompanied by high rates of incomplete beta-oxidation, which encourages intramitochondrial accumulation of acyl-CoAs and leads to subsequent mitochondrial failure (Koves et al. 2008). Intracellular pH drops in fatty acid exposed cells. This decrease in intracellular pH accompanies with the fatty acids' transfer across fat cell membranes following induction of lipolysis. After un-ionized, hydrophobic fatty acids diffuses across the cell membrane, they are re-ionized (Civelek et al. 1996). Excess FFAs also increase DAG, which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2) through phosphokinase C (PKC)-dependent pathways (Inoguchi et al. 2000). In high fat-fed murine hearts, suppression of autophagosome clearance and the activation of NOX are observed simultaneously. Actually, autophagy is a crucial catabolic process involved in maintaining energy and organelle homeostasis through degradation and recycling of organelles such as mitochondria or endoplasmic reticulum. Palmitate-induced NOX2 activation is dependent on the activation of classical PKC. In this respect, diminished autophagic turnover is a novel mechanism linking lipotoxicity with a PKCbeta-NOX2-mediated impairment in pH-dependent lysosomal enzyme activity (Jaishy et al. 2015; Quan et al. 2012). NOX2-derived ROS may promote FFA-induced dysfunction of pancreatic beta-cell through JNK pathway (Yuan et al. 2010). Indeed, FFAs can increase the formation of autophagosomes. Actually inhibition of autophagic degradation is accompanied by induction of autophagosome formation (Las et al. 2011). Beta-cells exposed to fatty acids show accumulation of abnormal autophagosomes. In this case, suppression of lysosomal gene expression contributes to the impairment of autophagic turnover (Las and Shirihai 2010). Suppression of autophagy enhances fatty acid-induced apoptosis. While unsaturated fatty acid promotes the formation of triglyceride-enriched lipid droplets and induces autophagy, saturated fatty acid is poorly converted into triglyceride-enriched lipid droplets and induces lipoapoptosis (Mei et al. 2011). It has been confirmed that autophagy deficiency in beta-cells could be a factor in the progression from obesity to diabetes due to an inappropriate response to obesity-induced endoplasmic reticulum stress (Quan et al. 2013).

Accumulation of fatty acids in the peripheral tissues alters the composition of membrane phospholipids due to development of inflammation, oxidative stress, lipid peroxidation. The higher toxicity of saturated or trans fatty acids seems to be the consequence of a blockade in triglyceride synthesis (Zámbó et al. 2013). Inappropriate accumulation of excess lipid in non-adipose tissues is associated with a chronic inflammatory response which is characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways (Wellen and Hotamisligil 2003). As a consequence, FFA-mediated lipotoxicity causes cellular stresses and inflammation by impairing normal cell signaling that may lead to apoptotic cell death (Unger 2003b). Thus increase in circulating levels of nutritional fatty acids in obesity activate toll-like receptor 4 (TLR4) signaling in adipocytes and macrophages and induce inflammatory signaling in adipose cells or other tissues and macrophages (Shi et al. 2006). TLR4 in macrophages and Kupffer cells participates in a sensing mechanism facilitating fatty acid-induced inflammation and IR (Diehl 2002). Excess lipid accumulation and abnormal energy metabolism lead to an overburdened endoplasmic reticulum. Increase in the synthetic activity of endoplasmic reticulum disrupts the normal folding of proteins and activates the unfolded protein response that is known to induce stress response pathways (de Luca and Olefsky 2008). Triggering of the endoplasmic reticulum stress by lipotoxic concentrations of saturated FFAs, subsequently leads to induction of the apoptotic transcription factor C/EBP-homologous protein (CHOP) and increases the percentage of apoptotic cells. Co-treatment with alpha-lipoic acid abolishes saturated FFA-induced lipoapoptosis by stimulating the nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Eventually Nrf2 eliminates the FFA-induced oxidative stress by activating antioxidant enzymes (Valdecantos et al. 2015). The capacity of a cell to manage oxidative stress is primarily mediated through antioxidant respon-

sive elements (AREs), which are largely under the control of the transcription factor Nrf2 (Jaiswal 2004). Under the basal condition, Nrf2-dependent transcription is repressed by a negative regulator Keap1. When cells are exposed to oxidative stress, Nrf2 escapes Keap1-mediated repression and activates ARE-dependent gene expression (Zhang 2006). It is well-known that mitochondria are the major ROS generating sources. Fatty acids accumulating in the vicinity of mitochondria are vulnerable to ROS-induced lipid peroxidation. Later, these lipid peroxides could have lipotoxic effects on mitochondrial DNA, RNA and proteins of the mitochondrial machinery and provoke mitochondrial dysfunction (Abdul-Ghani et al. 2008). If fatty-acid supply exceeds the oxidation rate, fatty acyl CoA might accumulate in the mitochondria and limit further fatty acid beta oxidation because of lack of free CoA. Acyl CoA is hydrolyzed within the mitochondria to fatty acid and free CoA. The fatty-acid anions are exported to the cytosol by uncoupling protein (UCP)3, allowing rapid fatty acid beta oxidation when the increased formation of fatty acids are protonated and flipped into mitochondria. Eventually, fatty acids accumulate up to ten-fold because of the pH gradient. They cannot be metabolized because of the lack of matrix acyl CoA synthases and may be toxic. UCP3 could lower the matrix concentration more than 1000-fold by transporting the fatty-acid anions. UCP2 and UCP3 increase the proton conductance of the mitochondrial inner membrane, but only when they are activated by products of ROS metabolism (Brand and Esteves 2005). While basal proton-leak depends on the fatty-acyl composition of the mitochondrial inner membrane and the presence of adenine nucleotide translocase, inducible proton-leak is controlled with UCPs (Divakaruni and Brand 2011). Fatty acids induce a conformational change in UCP1. Furthermore, saturated fatty acids accelerate the rate of enzymatic proteolysis of UCP1. The altered kinetics of both processes indicate that fatty acids change the conformation of UCP1, reconciling the apparent discrepancy between existing functional and ligand binding (Divakaruni et al. 2012). Free fatty acids operate as natural ‘mild uncouplers’ and prevent the transmembrane electrochemical proton potential difference from being above a threshold critical for ROS formation by complex I (Korshunov et al. 1998). On the other hand, due to their protonophoric action on the inner mitochondrial membrane, “mild uncoupling effect”, FFA strongly decreases ROS generation in the reverse mode of electron transport (Schönfeld and Wojtczak 2008). Both ROS and glutathionylation activates and deactivates UCP3-dependent increases in non-phosphorylating respiration. Increased cellular ROS levels coincide with UCP3 deglutathionylation. UCP3 deglutathionylation activates UCP3-mediated uncoupling and further decreases ROS emission. In this case Cys(25) and Cys(259) are the major glutathionylation sites on UCP3 (Mailloux et al. 2011). Moreover, mitochondrial uncoupling proteins works as carriers of fatty acid peroxide anions. UCP3 translocates fatty acid peroxide anions, which accumulate on the matrix side of the mitochondrial inner membrane, to outer leaflet and protects the mitochondria from oxidative damage. The metabolic pattern of matrix is much more complicated and important for the cell than that of the intermembrane space. Therefore, UCP-mediated transfer of fatty acid peroxides from the inner to outer leaflet has favorable biological effects (Goglia and Skulachev 2003). As mentioned above, mitochondrial uncoupling proteins are important regulator of mitochondrial ROS production. Thus, UCP3 is activated by lipid peroxides. Nevertheless, it removes anions and/or peroxides from the mitochondrial matrix, thereby specifically protects fatty acids from ROS-induced oxidative damage (Hoeks et al. 2006). At the same time, UCP3 could have a detoxification role, removing lipid peroxides from the matrix side of the inner mitochondrial membrane (Goglia and Skulachev 2003).

5 Lipid Droplets and Mitochondria

Neutral lipids, including triacylglycerols and cholesterol esters are stored in lipid droplets which are ubiquitous organelles. Lipid droplets-binding protein, perilipin, plays a critical role in

determining the characteristics of lipid-droplets (Kuramoto et al. 2012). Lipotoxicity is not only dependent on the presence of fat in non-adipose tissues, but also due to maintaining lipid homeostasis and metabolism of lipid droplets. During fasting, adipose tissue lipolysis is necessary for energy demands of non-adipose tissues (Jacob 1987). In conditions of chronic excess fatty acids which are transiently packed into lipid droplets in the form of triacylglycerols or cholesterol esters (Wang and Sztalryd 2011). Lipid excess packaging into lipid droplets can be seen as an adaptive response to fulfilling energy supply without hindering mitochondrial or cellular redox status and keeping low concentration of lipotoxic intermediates (Aon et al. 2014). In fact, adipocyte lipid droplets may play an important role in lipid homeostasis by providing the transient storage of fatty acid in the form of TGs. Thus, the formation of toxic lipid intermediates and their cellular toxicity could be prevented. In this case perilipin coating the lipid-droplets surfaces have an important function for the regulation of lipid stores (Wang and Sztalryd 2011). Lipid droplets in turn prevent excess ROS production by sequestering fatty acid from oxidation and hence suppress oxidative burden (Kuramoto et al. 2012). Perilipin 3, 4 and 5 bind to more transient pools of lipid droplets, while perilipin 1 and 2 associate with more constitutive pools of lipid droplets (Kovsan et al. 2007). Perilipin 5 may play a part in protection against cellular lipotoxicity by transiently entrapping bioactive lipids in lipid droplets (Wang and Sztalryd 2011). Perilipin 5 regulates oxidative lipid droplets hydrolysis and controls local fatty acid flux to protect mitochondria against excessive exposure to fatty acid during physiological stress (Wang et al. 2011). Mitochondria are in close physical interaction with perilipin 5-coated lipid droplets. Mitochondrial dysfunction provokes prominent lipid accumulation and tissue-specific metabolic disturbances in humans (Zehmer et al. 2009). Substantially perilipin 5 inhibits hydrolysis and stabilizes the lipid droplet. On the one hand palmitate accumulates into triglycerides and on the other hand mitochondrial utilization of palmitate is decreased. In PKA-stimulated state, inhibition of lipid droplets hydrolysis is abolished. Fatty acids are released from lipid droplets and undergone beta-oxidation in mitochondria. There are physical and metabolic links between lipid droplets and mitochondria. Consequently, perilipin 5 protects the mitochondria against fatty acids alterations by regulating lipid droplets' hydrolysis and local fatty acids' flux (Wang et al. 2011). Conversely, perilipin-null mice showed increased beta-oxidation in muscle, liver, and adipose tissue resulting from a coordinated regulation of the enzymes, UCPs-2 and -3 involved in beta-oxidation. The increased beta-oxidation can remove the extra FFAs created by the constitutive lipolysis (Saha et al. 2004).

6 Lipotoxicity and Insulin Resistance

Accumulation of intramuscular lipid due to insufficient mitochondrial fatty acid oxidation may be a causative factor in the development of IR (Hegarty et al. 2003). Insulin-resistant individuals have a reduced rate of fat oxidation compared with insulin-sensitive individuals (Kelley and Simoneau 1994), and decreased mitochondrial oxidative capacity of fat leads to an increase in intracellular fat content. Lowell and Shulman suggested that a mitochondrial defect in insulin-resistant individuals could lead to an increase in intramyocellular fat content (Lowell and Shulman 2005). Elevation of FFA concentrations in insulin-sensitive subjects causes a decrease in insulin sensitivity (Belfort et al. 2005). On the other hand developing IR with aging causes an increase in the intracellular fat content, which is associated with a 40% reduction in mitochondrial oxidative phosphorylation activity (Petersen et al. 2003). In humans dysregulated insulin action has been linked with an increased uptake of fatty acids into muscle, suggesting that an increased availability of fatty acids contributes to excess muscle lipid accumulation (Bonen et al. 2004).

Various hypotheses are proposed in order to clarify the adiposity-associated IR. The predominant paradigm used to explain this link is the portal/visceral hypothesis in which visceral depots

lead to increased FFA flux and inhibition of insulin action via "Randle's effect" in insulin-sensitive tissues (Smith and Ravussin 2002). The competition between glucose and fatty acids for their oxidation and uptake in muscle results in impairment of glucose metabolism by fatty acid oxidation. This condition is known as Randle Cycle which is also referred to as "fatty acid syndrome", involves a short-term inhibition of glucose transport and phosphorylation by fatty acids (Hue and Taegtmeyer 2009). In accordance with Randle's hypothesis fat produces proportional inhibitions of insulin-stimulated glucose uptake and of intracellular glucose utilization. Fatty acid could potentially be responsible for a large part of the peripheral IR (Boden and Chen 1995). In contrast to Randle's hypothesis increased concentrations of plasma fatty acids induce IR in human skeletal muscle through inhibition of glucose transport activity. This may be as a result of subsequent decrease in insulin receptor substrate-1 (IRS-1)-associated PI3K activity (Dresner et al. 1999). Later on McGarry et al. demonstrated a reasonable mechanism for the glucose-induced inhibition of fatty acid oxidation in which malonyl-CoA signals glucose utilization, also controls LCFA entry and oxidation in the mitochondria (McGarry et al. 1977). Recently Hue et al. proposed a mechanism, while the cytosolic accumulation of citrate inhibits 6-phosphofructo-1-kinase (PFK-1) (Garland et al. 1963); it regenerates acetyl-CoA, which turns into malonyl-CoA by ACC. Subsequently malonyl-CoA inhibits carnitine palmitoyltransferase (CPT) I and the entry of LCFA moieties into mitochondria are inhibited. In brief, malonyl-CoA prevents fatty acid oxidation and favors fatty acid esterification (Hue and Taegtmeyer 2009). This condition has been identified as glucose-fatty acid cycle. The concentrations of malonyl-CoA depend on the balance between the activities of ACC and malonyl-CoA decarboxylase (MCD) (Young et al. 2001). Inhibition of MCD increases malonyl-CoA and promotes glucose utilization and limits LCFA oxidation (Hue and Taegtmeyer 2009). Expression of ACC and MCD under the control of sterol regulatory element-binding protein (SREBP-1c and PPAR-alpha, respectively (Campbell et al. 2002; Young et al. 2001). It was shown that insulin activates the transcription factor SREBP-1c, which enhances transcription of genes required for fatty acid and triglyceride biosynthesis, most prominently ACC and fatty acid synthase (Brown and Goldstein 1997).

On the other hand, failure in the development of adequate adipose tissue mass due to ectopic storage of lipids or increased fat cell size, divert excess lipid into liver, skeletal muscle and the pancreatic insulin-secreting beta cells. Virtually ectopic fat deposition is the result of additive or synergistic effects including increased dietary intake, decreased fat oxidation and impaired adipogenesis. In this respect "acquired lipodystrophy" hypothesis creates a link between adiposity and IR (Heilbronn et al. 2004). Enlarged fat cells diminish capacity to store fat and are resistant to the antilipolytic effect of insulin. Chronically increased plasma fatty acids induce hepatic and muscle IR (DeFronzo 2004). When adipocytes exceed their storage capacity, fat begins to accumulate in non-adipose tissues which consist of specific metabolites that inhibit insulin signal transduction. IR is associated with enhanced Ser/Thr phosphorylation of IRS-1 and IRS-2, which impairs their interaction with the juxtamembrane region of the insulin receptor (Paz et al. 1997). Increase in the serine phosphorylation of IRS-1 at Ser(307) site by NEFAs could be one of the mechanisms leading to a decrease in IRS-1 tyrosine phosphorylation, PI3K activity and glucose transport (Le Marchand-Brustel et al. 2003). IRS-1 serine/threonine phosphorylation may mediate the desensitization of insulin signaling by stimulating the subcellular redistribution of IRS-1 and sensitizing IRS-1 to the action of the proteasome (Pederson et al. 2001). The impairment of insulin signaling by phosphorylation of IRS on serine and threonine residues, contributes to IR. In contrast to tyrosine phosphorylation, the multisite serine and threonine phosphorylation of IRS both positively and negatively regulates insulin signaling as well as correlates with their subcellular re-localization and/or proteasome-mediated degradation (Copps and White 2012). Ubiquitin conjugation of IRS-1 is a prerequisite for insulin-induced IRS-1 proteasome degradation. Both

tyrosyl phosphorylation of IRS-1 and PI3K activation are needed to activate the IRS-1 ubiquitin-proteasome degradation pathway. Activation of this pathway during prolonged insulin exposure underlies the molecular mechanism of IR (Zhande et al. 2002). Moreover, subcellular re-distribution of IRS-1 is regulated by the mammalian target of rapamycin (mTOR)-dependent pathway and facilitates also proteasomal degradation of IRS-1 (Takano et al. 2001). Actually, tyrosine phosphorylation of IRS-1 and its binding to PI3K are critical events in the insulin signaling cascade leading to insulin-stimulated glucose transport. Elevated plasma fatty acid concentration is associated with reduced insulin-stimulated glucose transport activity as a consequence of altered insulin signaling through PI3K (Le Marchand-Brustel et al. 2003). High concentrations of plasma FFAs are involved in the etiology of obesity-associated IR. In particular palmitic acid markedly inhibits insulin-stimulated phosphorylation of IRS-1, and Akt. In this case ubiquitination of the key insulin signaling molecules facilitates their proteasomal degradation (Hirabara et al. 2010; Ishii et al. 2015). Palmitic acid -induced IR is ameliorated by inhibiting the de novo synthesis of ceramide, inhibitor kappa B (IκB)-alpha degradation or mTOR activation (Lam et al. 2011). IR can be caused in the peripheral tissues by either activating IκB kinase alpha/NF-κB (Cai et al. 2005) or endoplasmic reticulum stress (Ozcan et al. 2004).

Lipid signaling molecules can be derived from saturated fatty acids, and they include long-chain fatty acyl CoA, DAG, phosphatidic acid, triacylglycerol and ceramides. Amongst them, DAG, triacylglycerol, and ceramides are directly associated with IR (Cooney et al. 2002; Nagle et al. 2009; Schmitz-Peiffer 2000). When adipocyte storage capacity is exceeded, lipid overflows into muscle and liver, and possibly into the beta-cells of the pancreas. Consequently, IR is exacerbated. Dysfunctional fat cells produce excessive amounts of IR-inducing and inflammation-provoking cytokines (DeFronzo 2004). With the development of a chronic inflammatory state, cytokines released from either adipocytes or from macrophages antagonize insulin action (Summers 2006). Hereby a chronic low-grade inflammation and an activation of the immune system are involved in the pathogenesis of obesity-related IR (Esser et al. 2014). Eventually, the accumulation of excess lipid and accompanied inflammation in adipose tissue and liver contribute to the development and progression of IR in peripheral tissues (Shoelson et al. 2007). As a result of imbalance between plasma FFA availability, fatty acid storage and fatty acid oxidation in the obese patient, muscle triacylglycerol-muscle oxidative capacity ratio is a marker of IR (van Loon and Goodpaster 2006). Decreased mitochondrial oxidative capacity of insulin-resistant young obese humans is independent of age (Phielix et al. 2014). Reactive lipid species such as fatty acyl CoA, DAG, and ceramides are important for the development of IR (Hajduch et al. 2001; Montell et al. 2001). Main lipid signaling molecules are derived from FFAs; DAG, which activates isozymes of the PKC family, and ceramide, which has several effectors including PKCs and a protein phosphatase (Schmitz-Peiffer 2000). Total cytosolic DAG correlates negatively with insulin sensitivity. Cytosolic DAG content is also associated with PKC activation and increases IRS-1 serine 1101 phosphorylation. Inhibition of insulin-stimulated IRS-1 tyrosine phosphorylation and Akt2 phosphorylation at serine 473 disrupts glucose transport into cells (Szendroedi et al. 2014). Eventual DAG levels are associated with reduction in both insulin-stimulated IRS-1 tyrosine phosphorylation and PI3K activity (Timmers et al. 2008). In addition to PKC, IκB and JNK can also be activated by acutely raising plasma FFA levels, which cause hepatic and peripheral IR (Boden et al. 2005; Hotamisligil 2005). Activation of PKC-theta leading to increased IRS-1 Ser307 phosphorylation. Activation of these serine/threonine kinases can interrupt insulin signaling by decreasing tyrosine phosphorylation of the IRS-1 (Yu et al. 2002). The metabolic actions of insulin are diminished by saturated fatty acids via inhibiting IRS/PI3K/Akt pathway. Eventually insulin-induced glycogen synthesis, glucose oxidation and lactate production are decreased. Both mitochondrial hyperpolarization and ATP generation decline as an evidence of mitochondrial dysfunction

(Hirabara et al. 2010). Indeed, DAG, fatty acyl CoA and ceramide associate with IR and serine phosphorylation of IRS-1 (Shulman 2000). In this case JNK-mediated phosphorylation of IRS-1 is a contributing factor during the development of IR (Sabio and Davis 2010). Only saturated FFAs cause a significant increase of mitochondrial ROS production, which correlates with concomitant mitochondrial DNA damage, mitochondrial dysfunction, JNK induction, apoptosis, and inhibition of insulin signaling. Blocking de novo synthesis of ceramide abolishes the effects of palmitate on mitochondrial ROS production, viability, and insulin signaling (Yuzefovych et al. 2010). In brief, it was suggested that lipid-induced IR is depend on the accumulation of lipid signaling molecules such as DAG and ceramide in ectopic tissues rather than inhibition of glycolysis and glucose oxidation. These lipid metabolites disrupt insulin-stimulated translocation of the GLUT4 glucose transporter (Muoio and Neufer 2012). JNK and PKC phosphorylate IRS-1, thus blunting its downstream targets PI3K and Akt. This results in down-regulation of glucose transporter (GLUT)-4 and IR. Impaired insulin sensitivity in the vascular endothelium leads to increased FFA oxidation, ROS formation, and subsequent activation of advanced glycation end products synthesis, PKC activation, protein glycosylation as well as down-regulation of prostaglandin I2 (PGI2) synthase activity. These events inhibit endothelial nitric oxide synthase (eNOS) activity thereby leading to endothelial dysfunction in obesity (Creager et al. 2003). In this case, the use of fatty acids instead of glucose for energy production enhances mitochondrial hydrogen peroxide production suggests that a localized catalase increase is needed to consume excessive mitochondrial hydrogen peroxide (Rindler et al. 2013b). Significant mitochondrial ROS formation during LCFA catabolism reflects a complex process involving multiple sites of ROS production as well as modified mitochondrial function. ROS are released not only on the matrix side of mitochondria but also on the cytosolic side of the inner membrane. ROS production is more sensitive to matrix levels of LCFA catabolic intermediates, indicating that mitochondrial export of LCFA catabolic intermediates can play a role in control of ROS levels. In addition, glutathione antioxidant system of muscle mitochondria is inhibited during LCFA catabolism (Seifert et al. 2010). The oxidized glutathione/2glutathione (GSSG/2GSH) couple is the most abundant redox couple in a cell (Schafer and Buettner 2001). NADPH is a key component in cellular antioxidation systems; and NADH-dependent ROS generation from mitochondria and NOX-dependent ROS generation are two critical mechanisms of ROS generation (Ying 2008). Palmitate strongly increases the cytosolic NAD^+/NADH ratio. FFA-induced ROS generation and apoptosis are accompanied by the decoupling of glycolysis and citric acid cycle fluxes leading to abnormal cytosolic redox states. The activation of citric acid cycle fluxes by palmitate are concomitant with reduced glycolysis and increased cytosolic NAD^+/NADH ratio (Noguchi et al. 2009).

Palmitate-induced dysregulation of mitochondrial oxidative metabolism is the primary cause of ROS accumulation and apoptosis. Unlike previously known, these metabolic alterations are independent of fatty acid beta-oxidation and precede the onset of oxidative damage or apoptosis initiation (Egnatchik et al. 2014).

As mentioned previously, the development of IR as a molecular consequence of enhanced mitochondrial hydrogen peroxide production is the response to increased reliance on fatty acids for energy production (Rindler et al. 2013a). On the other hand, stressing mitochondrial membrane potential through mitochondrial UCP5 causes a compensatory increase in mitochondrial UCP3. This leads to the depletion of the mitochondrial membrane potential and an increase in ROS production through the stressed electron transport chain. The stressed electron transport chain and ROS production induce activation of JNK1, which controls forkhead box protein O1a (FOXO1a) localization through dephosphorylating of Akt (Senapedis et al. 2011). UCP3 functions to export those fatty acids that cannot be oxidized from the mitochondrial matrix, in order to prevent deleterious fatty acid accumulation inside the matrix. In addition, UCP3 is increased

in patients with defective beta-oxidation and is reduced after restoring oxidative capacity (Schrauwen and Hesselink 2004). Inhibition of Akt phosphorylation at both Ser473 and Ser308 sites by palmitic acid occurs in a dose-dependent fashion. In this case palmitic acid not only inhibits insulin-stimulated Akt phosphorylation at Ser473, but also blocks insulin-stimulated phosphoinositide-dependent kinase-1 phosphorylation at Ser241. These evidences indicate that palmitic acid may impair the upstream insulin signaling (Wang et al. 2006). Lipid-activated signaling pathways are also likely to play an important role in interference with glucose-fatty acid cycle (Schmitz-Peiffer 2000). Saturated FFAs inhibit insulin stimulation of Akt that is a central mediator of insulin-stimulated anabolic metabolism (Chavez et al. 2003). Ceramide is the second messenger in the sphingomyelin signaling pathway. Excessive ceramide could contribute to the development of IR in peripheral tissues by two independent mechanisms. First, ceramide specifically blocks the translocation of Akt (PKB) to the plasma membrane. Second, ceramide inactivate Akt through acceleration of the enzyme dephosphorylation by activating protein phosphatase 2A (Chavez et al. 2003; Stratford et al. 2004). Ceramide also inhibits insulin-stimulated glucose transport in adipocytes. Similar reductions in hormone-stimulated translocation of the insulin-responsive GLUT4 and insulin-responsive aminopeptidase may occur (Summers et al. 1998). Ceramide and/or its derivatives, ganglioside GM3 and sphingosine, antagonize insulin signaling, induce oxidative stress, and inhibit glucose uptake and storage, and thus at least three mechanisms may initiate the molecular defects that underlie IR (Summers and Nelson 2005). Firstly, ceramide is a common molecular intermediate linking both glucocorticoids and saturated fatty acids to the induction of IR; secondly, different fatty-acid classes antagonize insulin-stimulated glucose uptake by distinct mechanisms distinguished by their dependence upon ceramide synthesis; and thirdly, inhibition of ceramide synthesis in obese rodents ameliorates IR and blocks the onset of diabetes. Consequently, inhibition of ceramide synthesis markedly improves glucose tolerance (Holland et al. 2007).

According to Morino et al. the main factor in the IR observed with obesity is the accumulation of LCFA derivatives and triacylglycerol in tissues due to impaired mitochondrial beta-oxidation (Morino et al. 2006; Ruderman et al. 1999). Accumulated fatty acids are used to synthesize DAG and ceramide. These signaling intermediates negatively regulate insulin effect. In this case increase in plasma fatty acid concentration results in an increase in intracellular fatty acyl-CoA and DAG concentrations, which results in activation of PKC-theta leading to increased IRS-1 Ser307 phosphorylation. This in turn leads to decreased IRS-1 tyrosine phosphorylation and decreased activation of IRS-1-associated PI3K activity resulting in free fatty acid-induced IR (Holland et al. 2007; Yu et al. 2002). Long-chain acyl-CoA molecules are converted to acylcarnitines by carnitine palmitoyltransferase 1 before mitochondrial import. MCD degrades malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase 1 (Rindler et al. 2013a).

While insulin binding induces tyrosine phosphorylation of the IRS, it also increases the activity of NOX in the plasma membrane. Increased concentration of H_2O_2 proximal to the IRS induces inositol trisphosphate (IP3) receptors and facilitates IP3-stimulated Ca^{2+} release, as well as impedes Ca^{2+} signals induced by insulin (Espinosa et al. 2009). In this case, total cytosolic DAG correlates negatively with insulin sensitivity. Acute induction of muscle IR is associated with a transient increase in total and cytosolic DAG content that is temporally associated with PKC-theta activation, increased IRS-1 serine1101 phosphorylation, and inhibition of insulin-stimulated IRS-1 tyrosine phosphorylation and Akt2 phosphorylation. In contrast, there are no associations between IR and alterations in muscle ceramide, acylcarnitine content (Szendroedi et al. 2014). Enhancing hepatic mitochondrial LCFA oxidation capacity in association with the carnitine palmitoyltransferase 1A expression can reverse IR and glucose intolerance in obese mice independent of hepatic steatosis (Monsénégo et al. 2012).

Carnitine acetyltransferase (CrAT) is a mitochondrial matrix enzyme that catalyzes the interconversion of acetyl-CoA and acetylcarnitine. Reduction in CrAT activity is accompanied by muscle accumulation of long-chain acylcarnitines (LCACs) and acyl-CoAs in obesity. Promoted mitochondrial influx of fatty acids resulted in accumulation of LCACs despite a pronounced decrease of CrAT-derived short-chain acylcarnitines. This lipid-induced antagonism of CrAT contributes to decreased mitochondrial pyruvate dehydrogenase activity and diminished glucose oxidation in the context of obesity (Seiler et al. 2014). Elevated palmitoyl carnitine concentrations inhibit electron transport chain activity and decrease the mitochondrial inner membrane potential. This deleterious action of FFA metabolites on mitochondrial substrate oxidation provides a potential link between the lipotoxicity, mitochondrial dysfunction, and IR (Abdul-Ghani et al. 2008).

Synthesis of cell membrane cholesterol and fatty acids begins with acetyl-CoA. Acetyl-CoA is converted to fatty acids and cholesterol through the 12 and 23 enzymatic steps, respectively. mRNAs encoding enzymes in these two pathways regulated by the SREBP family of membrane-bound transcription factors (Horton et al. 2002). SREBP serve as master regulators of lipid homeostasis by regulating synthesis of cholesterol, fatty acids, and triglycerides (Brown and Goldstein 2009). Newly synthesized SREBP is inserted into the membranes of the endoplasmic reticulum. During the cholesterol depletion, SREBP cleavage–activating protein (SCAP) transports the SREBP from the endoplasmic reticulum to Golgi apparatus. The NH2-terminal domain designated nuclear SREBP (nSREBP), translocates to the nucleus and binds to the promoter/enhancer regions of multiple target genes. If the cholesterol content of cells increases, SREBP cannot access to Golgi apparatus (Brown and Goldstein 1997). Golgi-to-endoplasmic reticulum transport of SCAP requires SREBP cleavage and that un-cleaved SREBP actively blocks recycling. SCAP not bound to SREBP cycled normally between the endoplasmic reticulum and Golgi, indicating that SCAP contains an endoplasmic reticulum retrieval signal (Shao and Espenshade 2014). SCAP deletion reduces lipid synthesis and prevents fatty livers despite persistent obesity, hyperinsulinemia, and hyperglycemia (Moon et al. 2012). Absence of insulin receptors selectively reduces IRS-2, but not IRS-1 phosphorylation, and the impairment of IRS-2 activation is associated with lack of insulin effects. Actually IRS-2 is more important than IRS-1 in mediating insulin action in liver (Rother et al. 1998). Despite the reduction in IRS-2, insulin continues to increase SREBP-1c, which activates the mRNAs encoding enzymes responsible for phosphorylation of glucose and its conversion to fatty acids. Glucose over-production-enhanced fatty acid synthesis interaction leads to setting up a vicious cycle (Shimomura et al. 2000). Activation of SREBP1 is highly dependent on the activity of insulin-induced genes 1 and 2 (Insig1 and Insig2), SREBP1 and Insig1 may be important for the process of human adipose tissue adaptation to excess lipid storage. The IR associated-obesity is dependent on the decreased Srebp1c mRNA expression in white adipose tissue rather than the amount of fat stored in morbidly obese individuals. Insig1 mRNA expression is decreased in the subcutaneous depot of morbidly obese individuals compared with obese patients and is further reduced in the presence of IR (Carobbio et al. 2013). The suppressor of cytokine signaling (SOCS)-3-induced IR occurs by decreasing insulin-induced IRS-1 tyrosine phosphorylation and its association with the regulatory subunit of PI3K (Emanuelli et al. 2001). Indeed, increased expression of SOCS-1 and SOCS-3 in liver and muscle of obese mice is associated with decreased tyrosine phosphorylation of IRS proteins (Ueki et al. 2004). Human SREBP-1c promoter was positively regulated by insulin and negatively regulated by STAT-3. SOCS-3-mediated attenuation of the STAT signaling pathway and resulting enhanced expression of SREBP-1c. Livers of morbidly obese individuals also exhibits enhanced expression of SOCS-3 protein and attenuated JAK/STAT signaling (Elam et al. 2010).

7 Conclusion

During prolonged nutrient excess or obesity, lipid influx can exceed the adipose tissue storage capacity, and results in accumulation of harmful lipid species at ectopic sites such as liver and muscle (Erikci Ertunc and Hotamisligil 2016). Consequently, large proportion of fatty acids enter into the mitochondria. In this case, ceramide and its derivatives antagonize insulin signaling, induce oxidative stress, and inhibit glucose uptake and storage. Finally, cessation of the bioenergetics and redox functions of mitochondria lead to cell death (Kroemer et al. 2007). For another aspect, leptin resistance is essential to permit accumulation of surplus calories into adipocytes, meanwhile, the lipo-oxidative action of leptin minimizes ectopic lipid accumulation. However, in advance stages of obesity peripheral organs also become leptin resistant and lipo-oxidative action could not protect the peripheral tissues from ectopic lipid accumulation (Unger and Scherer 2010). As noted, in this event, there is no single unifying mechanism that could be easily manipulated to protect the non-adipose tissue from detrimental effect of lipid spillover. Besides, a strategy may be to increase the capacity for lipid storage and prevent lipotoxicity, a second strategy to prevent lipotoxicity may be to increase the capacity of tissues to oxidize fatty acids (Medina-Gomez et al. 2007). The important point to be considered is; targeted proteins involved in deleterious action of FFA is simultaneously effective in many other intracellular signaling pathways (Bellini et al. 2015).

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