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Review Article

Lipotoxicity: Effects of Dietary Saturated and Transfatty Acids

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The ingestion of excessive amounts of saturated fatty acids (SFAs) and transfatty acids (TFAs) is considered to be a risk factor for cardiovascular diseases, insulin resistance, dyslipidemia, and obesity. The focus of this paper was to elucidate the influence of dietary SFA and TFA intake on the promotion of lipotoxicity to the liver and cardiovascular, endothelial, and gut microbiota systems, as well as on insulin resistance and endoplasmic reticulum stress. The saturated and transfatty acids favor a proinflammatory state leading to insulin resistance. These fatty acids can be involved in several inflammatory pathways, contributing to disease progression in chronic inflammation, autoimmunity, allergy, cancer, atherosclerosis, hypertension, and heart hypertrophy as well as other metabolic and degenerative diseases. As a consequence, lipotoxicity may occur in several target organs by direct effects, represented by inflammation pathways, and through indirect effects, including an important alteration in the gut microbiota associated with endotoxemia. Interactions between these pathways may perpetuate a feedback process that exacerbates an inflammatory state. The importance of lifestyle modification, including an improved diet, is recommended as a strategy for treatment of these diseases.

1. Introduction

Fat is an important component of the normal human diet. It is a source of energy and provides essential fatty acids and fat-soluble vitamins. However, several fatty acids in fats, especially saturated fatty acids (SFAs) and trans fatty acids (TFAs) may have adverse effects on human health [1–3].

In the human diet, SFAs are derived from animal sources, while TFAs originate in meat and milk from ruminant animals and result from bacterial biohydrogenation of unsaturated fatty acids in the rumen [4]. In addition, partial hydrogenation of unsaturated fatty acids in vegetable oils during the industrial production of certain foods produces TFA [5]. Small amounts of TFA are produced during the processes used to deodorize or refine vegetable oils to make the products more stable and robust and thus easier to handle or store [6, 7].

Most TFAs have physical properties similar to SFAs [8]. More specifically, monounsaturated TFA isomers with 18-carbon chain lengths (trans-18 : 1) are among the most predominant TFAs present in the human diet [9, 10]. It is well established that intake of SFA and TFA is a significant risk factor for cardiovascular diseases (CVD) as well as inflammation, insulin resistance, and obesity. These fatty acids also induce endothelial dysfunction and an unfavorable blood lipid profile, including increased LDL-c and decreased HDL-c levels [2, 11, 12].

High SFA and TFA intake, the typical dietary pattern of western populations, favors a proinflammatory status that contributes to development of insulin resistance. Roles for SFA and TFA intake have been demonstrated in several inflammatory pathways and result from imbalances in the highly interconnected lipid signaling pathways that contribute to disease progression in chronic inflammation, autoimmunity, allergy, cancer, atherosclerosis, hypertension, and heart hypertrophy as well as metabolic and degenerative diseases [13, 14].

The focus of this paper was to elucidate the influence and role of dietary SFA and TFA intake in lipotoxicity in the liver and the cardiovascular, endothelial, and gut microbiota

systems as well as in insulin resistance and endoplasmatic reticulum stress.

2. Insulin Sensitivity and Resistance

Insulin is an anabolic hormone that exerts several important metabolic effects. Insulin regulates glucose homeostasis at several levels, including decreasing hepatic glucose synthesis and increasing peripheral glucose uptake, primarily in muscle and adipose tissue. Moreover, this hormone stimulates lipogenesis and the synthesis of protein in hepatic and adipose tissues, while reducing lipolysis and proteolysis [15].

Events that occur after insulin binds to its receptor are highly regulated and specific and can be influenced by numerous factors such as the dietary composition, including the quantity and type of fatty acids [16, 17].

Although several mechanisms have been implicated in the development of insulin resistance [16], more studies are necessary to elucidate the link between the mechanisms of insulin resistance and fatty acid intake.

Increased lipid availability reduces insulin-stimulated glucose consumption in skeletal muscle. This effect is generally explained as a fatty acid-mediated inhibition of insulin signaling [15]. Moreover, in a recent investigation it was shown that a palatable hyperlipidic diet, rich with SFA, causes obesity and affects brain glucose metabolism in rats [18].

In a clinical study, short-term elevation of free fatty acids (FFAs) induced insulin resistance, which occurs primarily at the cellular level in skeletal muscle [17].

A chronic increase in plasma FFA levels is harmful as shown by the important effects of these dietary components in pancreatic beta cell lipotoxicity. Fatty acid derivatives can interfere with the function of these cells and ultimately lead to their death through lipoapoptosis [19].

Fatty acids in excess not only induce hepatic insulin resistance but also impair insulin clearance *in vitro* and *in vivo* in animals [20, 21] and humans [22]. This leads to the typical hyperinsulinemia observed in insulin-resistant states and in nonalcoholic fatty liver disease (NAFLD) [23, 24].

Several studies performed by our group have demonstrated that long-term interdisciplinary therapy reduces fat intake, in particular SFA, in obese adolescents. The intervention resulted in decreased visceral fat and tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels and increased levels of interleukin-10 (IL-10) and adiponectin, accompanied by a reduction in homeostatic model assessment-insulin resistance (HOMA-IR) and the occurrence of associated diseases [25–28]. In the context of these results, we proposed that the altered insulinemic status could be considered to play a key role in the development of several cardiometabolic risk comorbidities [12, 29–31].

In one review article, fatty acids were said to affect insulin secretion as a function of the chain length. Thus, insulin secretion increases as a function of longer carbon chains and decreases as a function of the degree of unsaturation. These findings suggest that SFA and TFA influence insulin resistance [32].

In an animal model, although TFA ingestion had no effect on fasting plasma glucose, insulin levels, or oral glucose tolerance, it significantly decreased insulin-stimulated glucose uptake in muscles compared to polyunsaturated fatty acids (PUFAs) [33].

In a cross-sectional study of individuals with high cardiometabolic risk, the association of TFA intake with insulin resistance was demonstrated. The authors speculated that TFA interferes with insulin signaling predominantly via intracellular kinases, which alter insulin receptor substrates [34].

Activation of intracellular kinases, such as inhibitor of nuclear factor-κB kinase (IKK) and c-Jun N-terminal kinase (JNK), alters insulin receptor substrates and decreases insulin sensitivity. Additionally, it is important to note that activation of transcription factors can contribute to reduced glucose uptake by the expression of proinflammatory cytokines, such as TNF-alpha and IL-6, causing impairment in the insulin receptor phosphorylation (Figure 3) [35].

The deleterious role of SFA in glucose and lipid metabolism has been previously shown. A partial explanation is that SFA increases production of cytokines such as TNF-α and IL-6 through hypertrophic adipocytes and infiltrating macrophages, and these cytokines cause the deterioration of insulin sensitivity [35].

Treatment of primary mouse hepatocytes and pancreatic cells with palmitic acid, an SFA, caused sustained JNK activation and insulin resistance. Moreover, the palmitic acid treatment inhibited glucose-induced insulin gene transcription. This effect may be mediated by interference of autocrine insulin signaling through the phosphorylation of insulin receptor substrates 1 and 2 at sites that interfere with their binding to activated insulin receptors [36]. It has been proposed that long-chain saturated fatty acids, such as palmitic acid, can trigger insulin resistance in both primary hepatocytes and pancreatic β-cells in a JNK-dependent manner. The JNK phosphorylation site on IRS-2 may be functionally equivalent to Ser-307 of IRS-1 (Figure 3). Moreover, JNK might also be involved in negative regulation of insulin synthesis or signaling within the pancreatic β-cells, the central site of blood glucose regulation. Thus, palmitic acid can be considered to be a potent activator of JNK in cultured hepatocytes and β-cells, leading to IRS-1 and IRS-2 Ser/Thr phosphorylation [36].

3. Hepatic Injury

The emergence of obesity has led to substantial prevalence of NAFLD. Worldwide, the prevalence of NAFLD ranges between 10% and 39%. However, the prevalence of NAFLD is increased in certain populations: the condition affects approximately 50% of diabetics, 57% to 74% of the obese, and up to 90% of morbidly obese people. Therefore, in the last decade, this disease has been recognized as an emerging clinical problem in obese patients [37, 38]. The pathogenesis of NAFLD is unclear, but there is evidence that insulin resistance, inflammation, and genetic and dietary factors, as

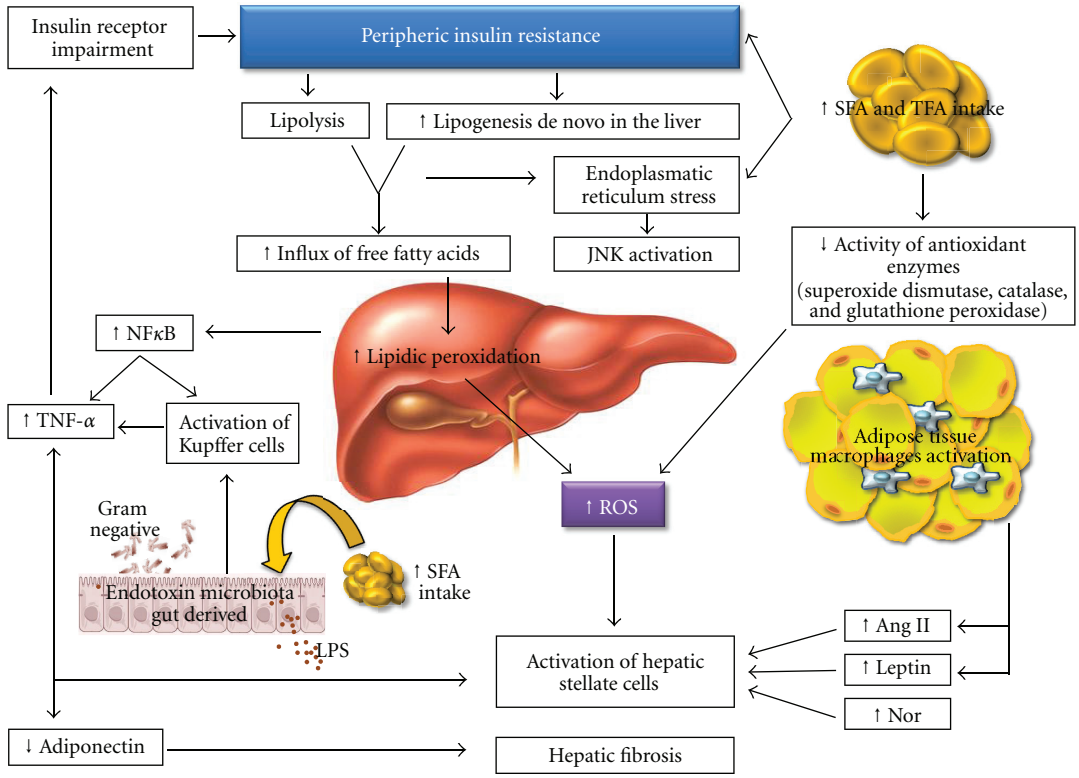

Figure 1: Schematic representation of SFA and TFA excess intake effects in hepatic injury and in endotoxemia. Ang II: angiotensin II, JNK: Jun N-terminal kinase, LPS: lipopolysaccharides, NF-κB: nuclear factor kappa-B, Nor: Noradrenaline, ROS: reactive oxygen species, SFA: saturated fatty acids, TFA: trans fatty acids, and TNF-α: tumor necrosis factor-alpha.

well as lifestyle, exert key roles in the development of NAFLD [39].

Several studies have revealed an association between obesity and NAFLD progression. In NAFLD patients, the adipocytes of the visceral tissue demonstrate elevated lipolytic activity, promoting a high influx of FFA into the portal vein [37, 38, 40]. Moreover, the literature shows an association between visceral adipocytes and the hepatic cellular inflammatory process [41]. It is believed that the physiopathology of NAFLD may be driven by several forms of hepatic injury. The first hypothesis involved in the development of NAFLD is related to insulin resistance. The influence of genetic and environmental factors can promote peripheral insulin resistance, which leads to increased levels of nonesterified fatty acids (NEFAs). Subsequently, a high influx of these fatty acids into hepatocytes promotes increased hepatic de novo lipogenesis. When the rate of lipogenesis exceeds the rate of β-oxidation of fatty acids and the exportation of VLDL, hepatic fat accumulation is observed (Figure 1) [40].

The exact mechanisms that promote the progression from steatosis to steatohepatitis have not yet been completely elucidated. However, it is clear that apoptosis can be a pathophysiologic marker of nonalcoholic steatohepatitis in some steatotic patients.

It has been proposed that the accumulation of FFA, especially saturated fatty acids, in the hepatocytes can promote apoptosis by diverse pathways. These may include ROS-induced stress that affects the mitochondrial membranes, endoplasmic reticulum, and lysosomes. Lipid peroxidation increases the levels of reactive oxygen species, which may be partially responsible for hepatocyte dysfunction [42] (Figure 1).

Apoptosis of the hepatocytes can occur via extrinsic or intrinsic pathways. The extrinsic pathway is induced by death ligands such as Fas (a key death receptor belonging to the tumor-necrosis-factor- (TNF-) receptor family) and TRAIL (TNF-related apoptosis-inducing ligand). In contrast, the intrinsic pathway of cell death is activated by the intracellular stress of membrane-bound organelles, such as ER, lysosomes, and mitochondria [43].

Saturated fatty acids activate complex intracellular pathways, including the activation of Toll-like receptor 4 (TLR-4), which subsequently stimulates TNF-α production. TNF-α consequently activates the JNK pathway [40], leading to the upregulation of the proapoptotic BH3-only protein PUMA (p53-upregulated modulator of apoptosis). PUMA then associates with BIM (Bcl-2-interacting mediator of cell death) to activate BAX, a proapoptotic protein of the BCL-2 (B-cell lymphoma 2) family. The end result of this pathway is mitochondrial dysfunction, activation of the caspase cascade, and cell death [43].

The TFA obtained in the diet accumulates in cellular triacylglycerols and phospholipids. Chronically excessive triacylglycerol accumulation in tissues such as the liver, muscle, and pancreatic beta cells leads to a protective response involving adaptation of the adipocytes, and this response

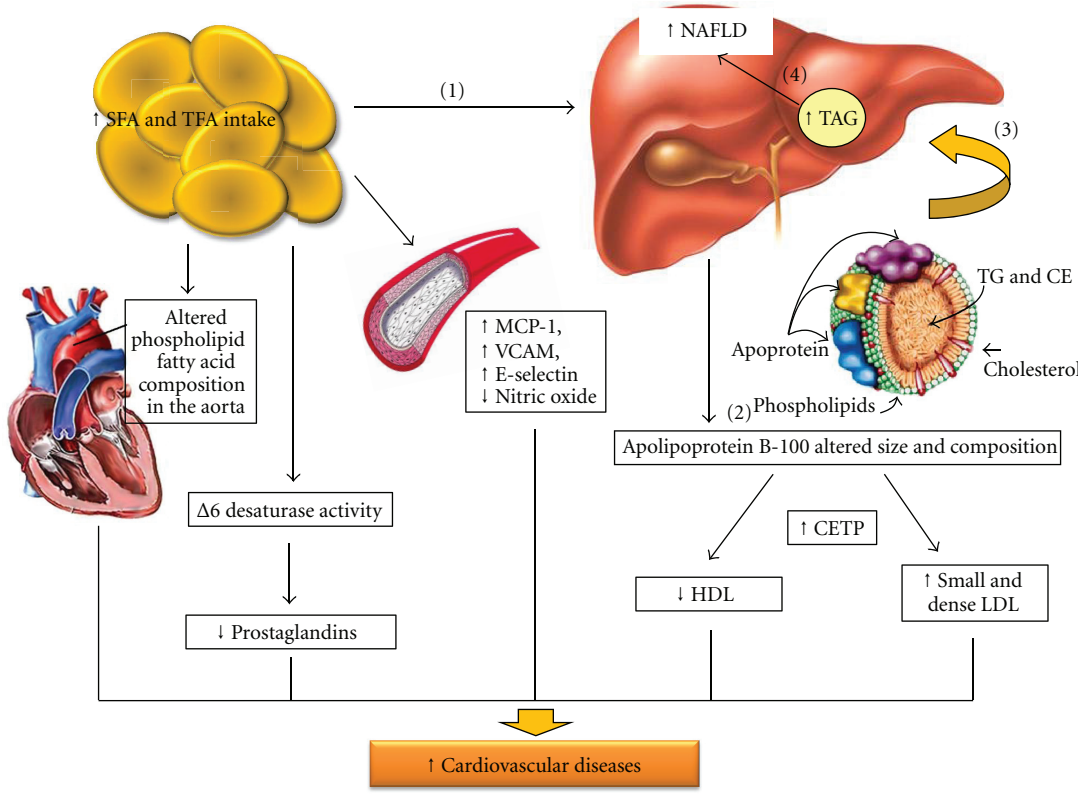

Figure 2: Schematic representation of SFA and TFA excess intake effects in hepatic injury, lipid metabolism, and cardiovascular risk. CE: cholesterol esterified, CETP: cholesteryl ester transfer protein, HDL: high density cholesterol, LDL: low density cholesterol, MCP-1: monocyte chemotactic protein-1, NAFLD: nonalcoholic fatty liver disease, SFA: saturated fatty acids, TFA: trans fatty acids, TG: triacylglycerol, and VCAM: vascular cell adhesion molecule.

includes activation of several inflammatory pathways that promote adipose tissue insulin resistance [34].

Toll-like receptors (TLRs) are classical pattern recognition receptors of the innate immune response. Two of these receptors, TLR4 and TLR2, are activated by SFA but inhibited by docosahexaenoic acid [44].

TLR4 activates both MyD88-dependent and -independent pathways. The MyD88-dependent pathway is initiated by the recruitment of TIRAP, an adaptor between the TIR domain of TLR-4 and MyD88, followed by activation of the IRAK family of protein kinases and subsequent phosphorylation of TRAF6. In the MyD88-independent pathway, the activation of TLR-4 requires the participation of TRAM to recruit TRIF, which then activates TRAF6 and TRAF3. TRAF6, directly or via TAK1, stimulates the IKK complex which promotes phosphorylation of IkB leading to activation of nuclear factor κB (NF-κB) (Figure 3) [45].

FFAs activate TLR-4 receptors in macrophages and adipocytes, which results in increased proinflammatory cytokine gene and protein expression [46]. Recently, it has been demonstrated that saturated fatty acids can induce TNF-α expression by macrophages by activating the MyD88-independent pathway [47].

TLR4 activation plays a central role in the inflammatory process, activating inflammatory cytokine gene transcription and also inducing endoplasmic reticulum stress. The activation of TLR4 by LPS induces a potent endoplasmic reticulum stress and unfolded protein response through the PERK/eIF2α and IRE1α/XBP1 pathways (Figure 4) [48]. NFkB, a transcription factor known as a mediator of immune and antiapoptotic responses, is activated by the accumulation of membrane proteins in the ER. Specifically, the accumulated proteins lead to the production of reactive oxygen intermediates, which activate NFkB by degradation of IkB. Recently, it has been proposed that the phosphorylation of eIF2a is required for the triggering of NFkB. In summary, the apoptotic process involves the activation of the gene for C/EBP homologous protein (CHOP), also known as growth arrest and DNA damage-inducible gene 153 (GADD153) (Figure 4); the activation of the cJUN NH2-terminal kinase (JNK) pathway, which is mediated by the formation of the inositol-requiring 1 (Ire1)-TNF receptor-associated factor 2 (TRAF2)-apoptosis signal-regulating kinase1 (ASK1) complex; and the activation of the ER-associated caspase-12. In humans, these apoptotic pathways eventually lead to the activation of caspase-3 [49].

The second hypothesis of NAFLD physiopathology refers to the release of fatty acids from dysfunctional and insulin-resistant adipocytes. This release causes lipotoxicity, mainly to visceral adipose tissue [40]. The visceral adipose tissue is infiltrated by inflammatory cells, including macrophages and other immune cells, which increase secretion of

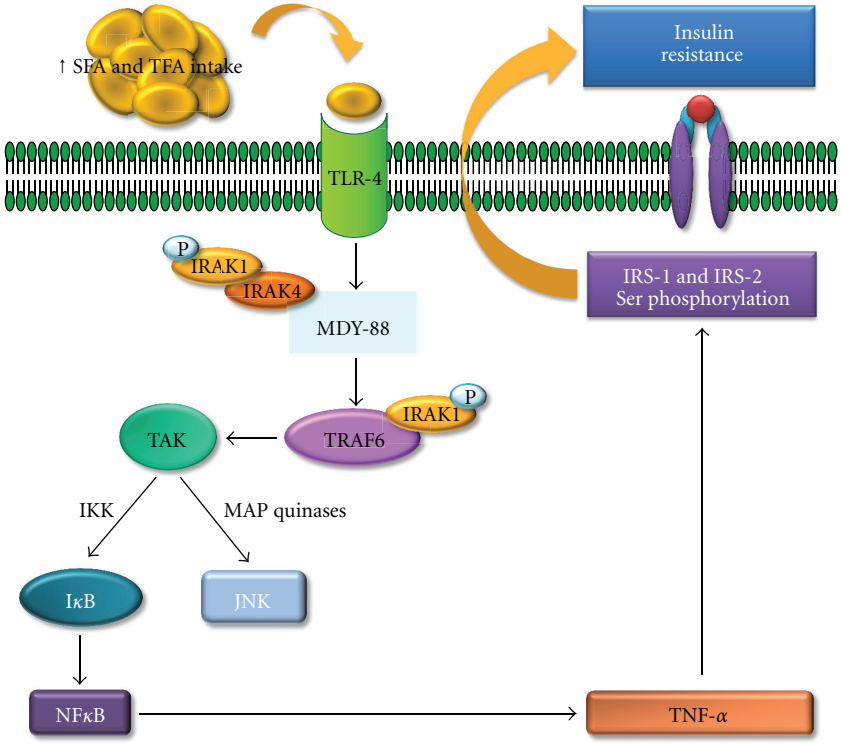

Figure 3: Schematic representation of SFA and TFA excess intake effects in the mechanisms of insulin resistance development. IKB: inhibitor of nuclear factor-κB, IKK: inhibitor of nuclear factor-κB kinase, IRAK-1: interleukin-1 receptor-associated kinase 1, IRAK-4: interleukin-1 receptor-associated kinase 4, IRS-1: insulin receptor substrate-1, IRS-2: insulin receptor substrate-2, JNK: Jun N-terminal kinase, MAP kinases: mitogen activated protein kinases, MDY-88: myeloid differentiation primary response gene (88), NF-κB: nuclear factor kappa B, P: phosphorus, TAK: thylakoid arabidopsis kinase, TLR-4: Toll-like receptor-4, TNF-α: tumor necrosis factor-alpha, TRAF-6: receptor-associated factor 6.

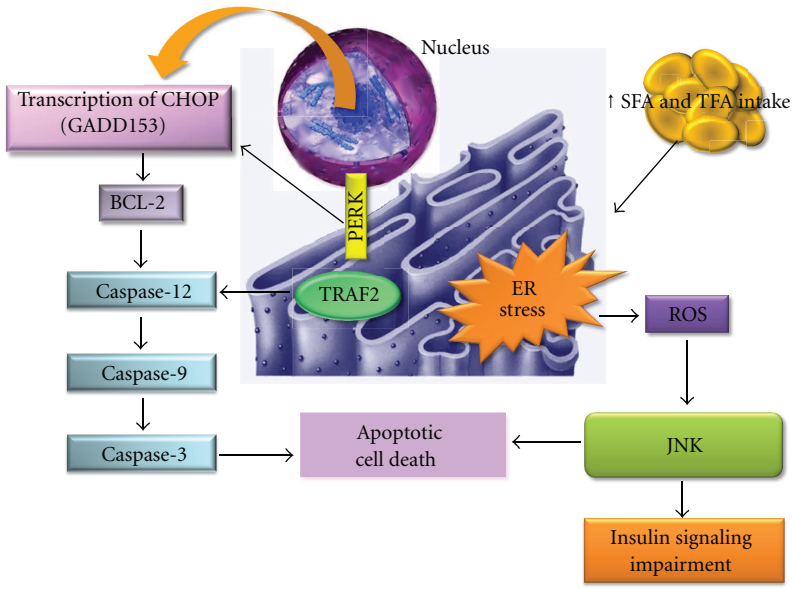

Figure 4: Schematic representation of SFA and TFA excess intake effects in endoplasmatic reticulum stress. BCL-2: B-cell lymphoma 2, CHOP: CCAAT/-enhancer-binding protein homologous protein, ER: endoplasmatic reticulum, GAAD 153: DNA damage-inducible gene 153, JNK: Jun N-terminal kinase, PERK: protein kinase RNA-like endoplasmic reticulum kinase, ROS: reactive oxygen species, SFA: saturated fatty acids, TFA: trans fatty acids, and TRAF2: TNF receptor-associated factor 2.

inflammatory adipokines such as leptin, IL-6, TNF-α, and angiotensinogen, as well as reducing secretion of adiponectin, an anti-inflammatory adipokine [50]. Insulin resistance of the visceral adipose tissue is associated with increased lipolytic activity, which increases the levels of FFA in the portal circulation, potentially resulting in hepatotoxicity (Figure 1) [40, 41].

Experimentally, it has been demonstrated that the decreased secretion of adiponectin in obesity alters lipid metabolism and insulin sensitivity in the liver. However, administration of recombinant adiponectin to adiponectin-deficient obese mice fed a high-fat diet dramatically alleviated hepatomegaly, steatosis, and inflammation [51].

At the clinical level, adipose tissue insulin resistance contributes to type 2 diabetes mellitus and CVD. On the other hand, decreasing plasma FFA concentration by administration of acipimox, a nicotinic acid analogue that inhibits adipose tissue lipolysis, rapidly improves muscle insulin sensitivity [52].

The potential ability of FFA to alter skeletal muscle glucose metabolism was first proposed more than 50 years ago [53] and has been widely investigated. The FFA-induced effects on this tissue do not appear to be the result of the accumulation of intramyocellular lipids per se. Rather, skeletal muscle insulin resistance is closely correlated with the presence of a variety of toxic metabolites derived from incomplete oxidation of fatty acids, such as acylcarnitines and long-chain fatty acyl CoAs, ceramides, and/or diacylglycerols [54, 55].

Oxidative stress is believed to be an important factor in the development of NAFLD [56]. The importance of fatty acids in this process is clear from the observation that biological membranes adjust their composition according to the fatty acid content of dietary fat [57]. Dietary fatty acids can influence the susceptibility of cells to oxidative stress, perhaps due to changes in the fatty acid composition of the cellular membranes [58].

Intake of high levels of SFA is associated with increased lipid content in the liver [18, 59–61] and with liver dysfunction. This dysfunction is thought to be caused by an increase in the production in reactive oxygen species, which leads to damage of the hepatic mitochondria. In addition, SFA intake exceeding 10% of total energy promotes insulin resistance, which plays a key role in the development of NAFLD [62].

The association between high intake of SFA and cholesterol with NAFLD has been previously demonstrated [63, 64]. SFAs promote endoplasmic reticulum stress as well as hepatocyte injury. Accumulation of SFA in the liver leads to an increase in markers associated with endoplasmic reticulum stress, such as reactive oxygen species and caspase activation. These biomarkers are associated with liver dysfunction. Moreover, the positive correlation between SFA intake and insulin resistance, which plays a key role in the development of NAFLD, has been demonstrated. These correlations suggest that limiting SFA intake is a valuable nutritional strategy for the prevention and treatment of NAFLD [62].

Papandreou et al. [64] demonstrated that the SFA intake was directly proportional to the degree of hepatic steatosis. In their study, multiple regression analysis of factors associated with fatty liver showed that HOMA-IR and SFA were the most significant factors for this condition after adjustment for age, gender, and diet. We have also observed an association among SFA intake, NAFLD, and orexigenic neuropeptides [26].

Diets rich in fatty acids, mainly SFA and TFA, as well as carbohydrate-rich diets, favor an acute increase in insulin resistance independent of adiposity. High SFA intake may also promote steatohepatitis directly by modulating hepatic triacylglycerol accumulation and oxidative activity and indirectly by affecting insulin sensitivity and postprandial triacylglycerol metabolism [64].

In clinical studies performed by our research group, we showed a positive correlation between calories derived from SFA intake and visceral fat in NAFLD patients [23, 26]. These data suggested that composition of the diet exerts an important role in the development of NAFLD and its treatment and that it is essential to consider excessive SFA intake as a critical risk factor for development of NAFLD [26].

Another potentially important mechanism for lipotoxicity of TFA in the liver is their effects on hepatic antioxidant enzymes. Reactive oxygen species (ROS) form as natural byproducts from the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress, ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. Endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are essential to defend against ROS. The removal of reactive oxygen substances is accomplished by enzymatic and nonenzymatic reactions in biological systems. In enzymatic reactions, SOD converts superoxide anions to hydrogen peroxide (H_2O_2), and H_2O_2 can be rapidly degraded by CAT and GPx to H_2O [65–67].

High-fat diets can cause the formation of toxic intermediates that can inhibit the activity of antioxidant enzymes, resulting in the accumulation of O_2 radicals and H_2O_2, which subsequently form hydroxyl radicals [68, 69]. TFAs are associated with a decrease in the efficiency of the antioxidant-enzymatic system and therefore with the increase of oxidative stress in rat livers. TFA may impart their effect by enhancing intrinsic signaling mechanisms leading to a chronic, pro-inflammatory state. Consumption of diets high in TFA may induce long-term progressive changes in the antioxidant enzyme's activities [70].

Finally, another pathway by which excessive TFA intake could cause hepatic injury is through effects on lipid metabolism. *In vitro*, TFAs alter the secretion, lipid composition, and size of apolipoprotein B-100 (apoB-100) particles produced by hepatic cells [71] (Figure 2). Specifically, these cells fail to synthesize the apolipoprotein, which is required to package and export fat from the liver. Therefore, the liver accumulates triacylglycerol. In a study in which male Wistar rats were fed a high-fat diet including 20% fresh soybean oil diet, 20% oxidized soybean oil diet, or 20% margarine diet for 4 weeks, the highest inflammatory response in the liver was induced by the margarine diet. The authors demonstrated that oxidized edible oils fed to rats for four

weeks increased lipid peroxidation in the liver compared with rats fed nonoxidized oils. These results suggest that a strong relationship exists between the consumption of TFA in the oxidized oils and lipid peroxidation. This study provides evidence for a direct effect of TFA on liver dysfunction caused by disturbances in hepatic lipid metabolism. The resulting NAFLD is a key component of cardiometabolic risk. This evidence suggests that TFA may influence the risk factors for CVD [70].

Thus, the link between dysfunctional adipocytes and the liver involves several pathways that combine to promote the development of lipotoxic liver disease, a term that more accurately describes the pathophysiology of nonalcoholic steatohepatitis.

In fact, hepatic steatosis is considered to be a hepatic manifestation of cardiometabolic risk. This condition is associated with obesity, insulin resistance, hypertension, and dyslipidemia. Clinical studies corroborate the relationship between NAFLD and CVD. Specifically, obese NAFLD patients were shown to have a greater intima-media thickness (IMT), a subclinical marker of the atherosclerotic process, compared to non-NAFLD patients [72]. In this context, our research group performed a study that involved one year of interdisciplinary intervention in obese adolescents. The results indicated that the improvement of HOMA-IR was an independent predictor of carotid IMT changes in this population [30]. As previously discussed, hyperinsulinemia from increased insulin secretion and decreased insulin clearance correlates with the severity of hepatic steatosis, and chronically elevated plasma insulin levels may promote atherogenesis [73, 74]. Hyperglycemia, *per se*, and the typical atherogenic dyslipidemia in NAFLD driven by oversecretion of VLDL are established factors for CVD. However, whether NAFLD and CVD are mechanistically related or merely both associated with lipotoxicity remains to be established.

4. Cardiovascular Risk

Studies suggest multiple possible mechanisms that might mediate the association of TFA with CVD. Three main pathways for these physiological effects have been proposed: serum lipid concentrations, systemic inflammation, and endothelial cell function [3, 75].

Consumption of industrial TFA increases the blood concentrations of low-density lipoprotein (LDL), triacylglycerols, and Lp(a) lipoprotein while decreasing the levels of high-density lipoprotein (HDL) and reducing the particle size of LDL cholesterol. Furthermore, consumption of TFA can increase the ratio of total cholesterol to HDL cholesterol, a powerful predictor of CVD risk [3, 76]. Thus, TFAs have markedly adverse effects on serum lipid profiles.

As described previously, there is an important relationship between intake of TFA and incidence of CVD. However, the effects exceed those predicted by the changes in serum lipids alone, suggesting that TFAs influence other risk factors for CVD [77]. Specifically, in addition to their effects on lipid/lipoprotein profiles, TFA consumption is known to influence multiple risk factors including increased systemic inflammation [78], increased thrombogenesis, and reduced endothelial function [2], all of which, in combination or individually, contribute to increased cardiovascular risk. Experimental studies suggest that TFAs exert their multiple effects by influencing metabolic and signaling pathways in hepatocytes, monocytes, adipocytes, and endothelial cells. The precise molecular pathways through which TFAs influence these cell types are not well established [77].

The effect of TFA on systemic inflammation can be partially explained by the influence of these fatty acids on the prostaglandin balance. The effects on these processes can influence thrombogenesis and impair the activity of Δ desaturase, the enzyme responsible for the conversion of linoleic acid to arachidonic acid and other n-6 PUFA. Thus, this inhibition alters essential fatty acid metabolism [79]. Moreover, in an animal model of excess TFA consumption, changes in the phospholipid fatty acid composition in the aorta were observed [80] (Figure 2). TFAs have been associated with the activation of systemic inflammatory responses, including substantially increased levels of IL-6, plasminogen activator inhibitor-1 (PAI-1), TNF-α, TNF receptors, and monocyte chemoattractant protein-1, and with increased levels of several markers of endothelial activation, such as soluble intercellular adhesion molecule 1, soluble vascular-cell adhesion molecule 1, and E-selectin [2, 3, 81] (Figure 2).

In controlled trials, however, TFA did not increase all inflammatory markers [78, 82]. Oxidative stress may also explain the high risk of CVD associated with industrial TFA intake [83].

Oxidative stress induced by free radicals has been associated with the development of several diseases including CVDs, most likely through a vascular proinflammatory response [84]. However, further research is necessary to fully elucidate the implications of the effects of TFA on some markers of oxidative stress. Although the possible mechanisms that link TFA and oxidative stress are unknown, efforts to eliminate partially hydrogenated oils from the diet remain necessary and important to reduce the burden of CVD [70].

The third pathway linking TFA and CVD refers to the possible influence of TFA on endothelial cell function. Endothelial nitric oxide synthase (eNOS) synthesizes nitric oxide (NO) in response to many stimuli, such as fluid shear stress and insulin. These stimuli increase NO production in endothelial cells through an insulin receptor substrate-1 (IRS-1-) and phosphatidylinositol 3-kinase (PI3-kinase-) dependent pathway that causes phosphorylation of endothelial nitric oxide synthase (eNOS) by Akt [85].

A review of the influences of fatty acids on endothelial cell function suggested that increased ingestion of fatty acids impairs endothelial cell insulin signaling and NO production through the activation of the IKK/NF-κB pathway. Furthermore, an experimentally induced elevation of the concentration of plasma FFA in humans alters endothelial function [46].

It has also been shown that the dietary SFA palmitate attenuates endothelial insulin signaling and NO production by first activating NF-κB signaling, which results in a reduction in IRS-1/pAkt/peNOS signaling [77].

The reduction of SFA intake is considered a primary goal for decreasing the risk of CVD. A low SFA diet was demonstrated to be associated with the reduced progression of coronary atherosclerosis [86, 87].

The effect of SFA intake on the plasma lipid risk factors and effects on CVD are similar to those described for TFA intake. However, SFA ingestion is particularly associated with activation of the TLR pathways.

In a clinical investigation, TLR-4 and TLR-2 expression and activity were increased in the monocytes of patients with cardiometabolic risk. The pathways regulated by these receptors could contribute to the patients' high risk for CVD [88].

5. Endoplasmic Reticulum Stress

Lipid peroxidation is defined by a biochemical cascade that results in oxidative degradation of PUFA. When the lipid peroxidation occurs in biological membranes, it causes impaired membrane function and structural integrity, decreases in membrane fluidity, and inactivation of several membrane enzymes [89]. Niu et al. [90] reported that phospholipids derived from TFA had a higher membrane cholesterol affinity than their *cis*-analogues. Thus, TFA ingestion could alter cell membrane structure, organization, and composition in an ROS-mediated manner.

A recent animal experiment indicated that TFA reduced the membrane fluidity of fat cells and impaired cell function. The suggested mechanism involved production of additional reactive oxygen species associated with the increase in lipid peroxidation in the groups fed the TFA diet [91]. A high-fat diet induces endoplasmic reticulum stress (ER), which activates IKK and JNK, thereby impairing insulin signaling [92].

Recent evidence suggests that lipotoxicity in hepatocytes involves ER stress and JNK-mediated apoptosis [93, 94].

Disturbances in the normal functions of the ER lead to an evolutionarily conserved cell stress response, the unfolded protein response, which is aimed initially at compensating for damage but can eventually trigger cell death if ER dysfunction is severe or prolonged. Although the mechanisms by which ER stress leads to cell death are not completely understood, some of them have been described in the literature. A study of mice deficient in caspase-12 showed that while the cells of these mice were resistant to ER stress-induced apoptosis, apoptosis of the cells occurred normally in response to other death stimuli [95]. Based on these data, it was proposed that other pathways leading to cell death by ER stress should be explored. Increases in apoptotic proteins, such as BIM, BAK, and PUMA, were observed during ER stress, suggesting a connection between stress signals and the proapoptotic switch that occurs when cellular homeostasis is irreversibly altered, finally leading to cell death [96, 97]. The ER responds to the burden of unfolded proteins in its lumen by activating intracellular signal transduction pathways, generically termed the unfolded protein response (UPR). Another suggested mechanism is that the three UPR branches provide opposing signals and that the relative timing of their induction shifts the balance between cytoprotection and apoptosis as unmitigated ER stress persists. Specifically, IRE1 signaling attenuates upon prolonged ER stress, and PERK (protein kinase RNA-like endoplasmic reticulum kinase) signaling induces its own deactivation via GADD34 expression (Figure 4). Both pathways thus contain intrinsic timers that are likely to contribute to the life-or-death decision [98].

Important roles for ER-initiated cell death pathways have been recognized for several diseases, including hypoxia, ischemia/reperfusion injury, neurodegeneration, heart disease, and diabetes [99].

Studies suggest that cytokines, as well as elevated lipids, especially long-chain SFA, may induce ER stress in pancreatic β-cells and liver cells. SFA-induced β-cell death has been shown to be related to the activation of caspases [94, 100]. Elevated lipids also induce apoptosis in a number of cell types, suggesting that ER stress may be an early component of lipotoxicity [94].

A study of cultured H4IIE liver cells investigated the influence of SFA and TFA in the apoptosis process and the role of the ER stress-induced activation of caspases. The authors observed that SFA induced ER stress and increased both caspase-9 and caspase-3 activity (Figure 4). The authors hypothesized that saturation, *per se*, plays a role in lipotoxicity in liver cells [94].

6. Gut Microbiota

The human gut contains a massive number of microorganisms or microbiota. Several mechanisms have been proposed to link gut flora to obesity, including the role of the gut microbiota in increasing energy extraction from indigestible dietary polysaccharides [101] and elevating plasma lipopolysaccharide levels, resulting in chronic low-grade inflammation [102].

The intestinal flora exerts an important role in normal gut function and maintenance of health, and the dietary composition can influence the sequence and the nature of colonization. Cani et al. [103] found that a high-fat diet resulted in a significant change in the composition of the dominant bacterial populations within the gut microflora, including a decrease in the number of *Bifidobacteria*, Eubacterium, rectal *Clostridium coccoides* group, and *Bacteroides*, thus favoring an increase in the gram-negative to gram-positive ratio. This change in gut microflora composition was associated with a significant increase in plasma lipopolysaccharide (LPS) levels, fat mass, body weight gain, liver hepatic triglyceride accumulation, insulin resistance, and diabetes [103, 104]. In addition, de Wit et al. [105] observed that a high saturated fatty acid diet enhanced an overflow of dietary fat to the distal intestine, which affected the gut microbiota composition. This alteration was associated with obesity development and hepatic steatosis.

The gut microbiota of obese individuals or those consuming a high content of saturated fatty acids contains predominantly gram-negative bacteria rich in LPS. Toll-like receptors in the cell membranes recognize LPS in the circulation (endotoxemia) and activate specific kinases, which lead to insulin resistance. These pathways also activate NF-κB, which results in the expression of inflammatory genes. Similar to

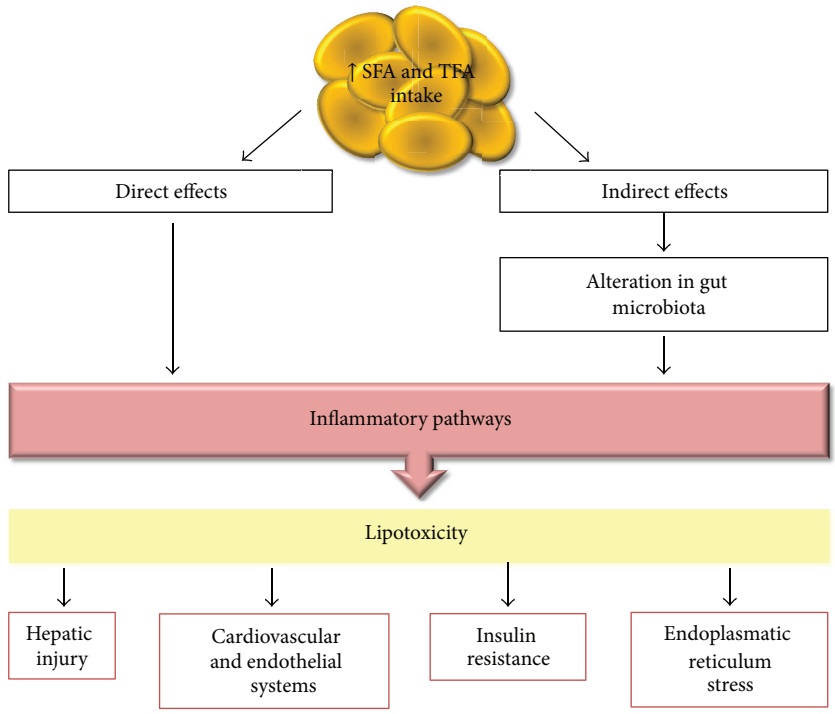

Figure 5: Schematic representation of SFA and TFA excess intake effects in the development of lipotoxicity in several target organs.

LPS, saturated fatty acids are also recognized by membrane receptors that trigger proinflammatory signaling pathways [102, 106].

Recently, our group demonstrated a positive correlation between plasma endotoxin concentration and both proinflammatory cytokines (especially IL-6) and insulin resistance in obese adolescents. Importantly, after long-term (one year) interdisciplinary therapy, endotoxemia, proinflammatory status, and insulin resistance were decreased [25]. These results showed the effectiveness of making lifestyle changes (i.e., nutritional modification) in reducing the proinflammatory state in obese individuals [107, 108].

7. Conclusion

These experimental and clinical findings indicate that excess intake of both SFA and TFA can promote lipotoxicity in several target organs by direct effects, represented by inflammatory pathways, and indirect effects, including important alterations in the gut microbiota with implications for the endotoxemia process (Figure 5). Interplay between these pathways perpetuates a feedback process in which an inflammatory state elevates the risk factors for diverse diseases.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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