Possible molecular mechanisms soy-mediated in preventing and treating nonalcoholic fatty liver disease

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Abstract

The aim of this review is to describe the molecular mechanisms of nonalcoholic fatty liver disease (NAFLD) and to present evidence regarding the mechanisms of soy-mediated therapeutic activity in preventing and treating NAFLD. NAFLD is induced by multiple metabolic pathways, including an increase in the release of fatty acids from the adipose tissue (lipolysis), insulin resistance (IR), and an increase in “de novo” fatty acid synthesis. Furthermore, NAFLD is correlated with a decrease in liver β-oxidation, an increase in oxygen free radical production, and an increase in pro-inflammatory cytokine production, which leads to an increase in liver fat and, subsequently, to tissue damage.

The bioactive compounds in soy can prevent and treat NAFLD by modulating lipid metabolism and regulating the expression of related transcription factors. Soy intake decreases the expression of sterol regulatory-element binding protein-1c (SREBP-1) and increases the expression of SREBP-2, which are transcription factors associated with the regulation of hepatic lipogenesis and reduction of cholesterol synthesis and absorption in the liver, respectively. Besides, interactions between soy components, such as standard amino acids, polyunsaturated fat, and the isoflavonoid-enriched fraction, are believed to improve fatty acid oxidation in the liver parenchyma by increasing the expression of peroxisome proliferator-activated receptor α (PPARα)-regulated genes, thus decreasing lipid accumulation in the liver. Therefore, including soy-derived foods in the diet as a therapeutic tool for patients with NAFLD might improve their clinical evolution.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a generic term used to describe several liver disorders involving lipid deposition in the hepatocyte cytoplasm of patients who do not consume excessive ethanol. These disorders range from liver steatosis to nonalcoholic steatohepatitis (NASH), which exhibits important histopathological features such as steatonecrosis, Mallory bodies, and fibrosis that could progress into cirrhosis and hepatocellular failure. Lifestyle changes such as a low-fat diet and physical exercise have been associated with an improvement in insulin resistance, liver enzymes and cholesterol serum levels in NASH patients. Studies have also shown that the amount of dietary fat influences the lipid content of the liver and that NASH can evolve into hepatocellular fibrosis and carcinoma.

Given that the high prevalence of obesity and metabolic syndrome (MS) increases the risk of developing hepatocellular damage, NAFLD must be acknowledged as an important public health problem. Individuals with an appropriate weight but increasing abdominal circumference and insulin resistance (IR) are also susceptible to NAFLD.

The prevalence of NASH in obese individuals is 19%; however, this rate increases to 50% in the severely obese, whereas it is only found in 3% of the non-obese population. Diabetes mellitus (DM) is another disease associated with NAFLD. The prevalence of DM in the American adult population is 7.8%, and about half of these patients exhibit NAFLD. The combination of obesity and DM may be an additional risk factor for fatty infiltration because 100% of severely obese individuals with DM exhibit moderate liver steatosis; 50% exhibit steatohepatitis, and 19% exhibit cirrhosis. These findings suggest that the physiopathology of NAFLD is related to excessive weight, inflammation, and IR.

Some studies have suggested a strong correlation between these two conditions in which IR is a common trigger. A study performed in non-diabetic individuals showed that liver fat was significantly greater in patients with MS compared to patients without MS, regardless of age, sex, or body mass index (BMI). The course of NAFLD depends synergistically on individual and environmental factors and can be established from the severity of the histopathological damage and progression into steatohepatitis or liver fibrosis. Although most individuals with NAFLD only exhibit liver steatosis, one study showed that 47% of patients with simple steatosis will develop NASH within 8 to 13 years and that 25 to 50% of patients with NASH will develop advanced fibrosis and cirrhosis.

An epidemiological study of 3,245 adult individuals showed that NAFLD is associated with elevated alanine aminotransferase (ALT) serum levels, obesity, DM, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia.

This review aims to describe the molecular mechanisms of NAFLD and to present data on the effect of soy-mediated therapeutic mechanisms on lipid metabolism, IR, reducing oxidative stress, and inflammation, which are all essential elements of the development of NAFLD.

Data search and selection strategy

A survey of the scientific literature was performed by searching electronic databases to identify international studies published between 2000 and 2011 that addressed the use of soy supplements and the progression of NFLD. The electronic databases accessed included Scientific Electronic Library On-line (SciELO), Latin American and Caribbean Literature on Health Sciences (Lilacs), and Medical Literature Analysis and Retrieval System Online (MedLine) of the National Library of Medicine. Search terms for titles or abstracts were soy protein, hepatic steatosis, fatty liver disease, non-alcoholic steatohepatitis, insulin resistance, and hepatitis. The search was restricted to articles published in English, and experimental and clinical studies were selected. An additional manual
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Pathogenesis

Multiple metabolic pathways can contribute to NAFLD, including an increase in the release of non-esterified fatty acids from the adipose tissue (lipolysis), IR, increased “de novo” synthesis of fatty acids (lipogenesis via gene transcription), and decreased β-oxidation in the liver. These conditions cause an increase in the production of reactive oxygen species (ROS), hypersecretion of leptin, which increases lipolysis, and hypersecretion of ghrelin, which increases food intake. Oxidative stress also promotes hyperstimulation of Ito cells, which produce collagen in the hepatic parenchyma, and consequent fibrosis and cirrhosis, which may progress into hepatocellular carcinoma.

Oxidative stress caused by excess ROS is significantly related to NAFLD progression. In a recent review, Koek et al. concluded that ROS levels produced at the mitochondrial, microsomal, peroxisomal, and endoplasmic reticulum play an important role in the progression from liver steatosis to NASH. Overload of free fatty acids (FFAs) induces the release of mitochondrial electrons during β-oxidation, resulting in an increase in the production of lipid peroxides and subsequent damage to hepatocyte plasma membranes, cellular proteins, and DNA. Moreover, enzymatic and non-enzymatic antioxidant systems are unable to prevent liver damage, thus inducing the onset of inflammation. The increase in pro-inflammatory cytokines also contributes to peripheral IR, with increased fatty infiltration of the liver parenchyma and consequent tissue damage.

Liver steatosis correlates with hyperproduction of glucose, VLDL, C-reactive protein (CRP), and coagulation factors, intra-abdominal fat accumulation, and the inflammation profile, as well as greater synthesis of tumor necrosis factor-α (TNF-α) and interleukin (IL) 1 and 6. NASH is characterized by diffuse fatty infiltration of the liver, ballooning degeneration, hepatocyte inflammation, and initial fibrosis. A study analyzing liver fatty acids and triglycerides of obese patients by chromatography showed that 59% of liver triglycerides came from non-esterified fatty acids (NEFAs); 26% came from “de novo” fatty acid synthesis, and 14.9% came from diet.

The onset of hepatocellular damage and NASH is explained by the two-hits theory. According to this theory, liver steatosis and IR appear first (first hit) as an adaptive mechanism or due to genetic predisposition. Thus, steatosis sensitizes hepatocytes to the action of free radicals, which induce oxidative stress in the liver tissue and thus cause tissue damage (second hit). However, recently, the multiple hits hypothesis suggest that inflammatory mediators derived from various tissues but especially from the gut and adipose tissue could play a central role in the cascade of inflammation, fibrosis, and finally tumor development. Endoplasmic reticulum stress and related signaling networks, (adipo) cytokines, and innate immunity are emerging as central pathways that regulate key features of NASH.

There is a strong association between NAFLD and IR in the visceral and liver adipose tissue. IR is caused by the inhibition of intracellular signaling pathways, which diminishes the cellular response to the action of insulin. In IR, visceral fat lipolysis occurs in combination with reduced fatty acid capture and oxidation by peripheral tissues. Consequently, the amount of circulating FFAs that reach the liver tissue increases.

To understand the mechanisms that establish IR, it is first necessary to understand how insulin permits glucose to enter the cell. A recent review described the primary insulin signaling pathways and showed that upon binding insulin, the insulin-tyrosine receptor is auto-phosphorylated and induces phosphorylation of tyrosine residues on insulin receptor substrates (IRSs). IRS-1 starts the glucose metabolism pathway. After being phosphorylated, it stimulates the phosphatidylinositol 3-kinase (P13K)-AKT/protein kinase B (PKB) pathway, which recruits glucose transporters (GLUTs) and allows glucose to enter the cell. Glucose primarily enters the cells via membrane protein-facilitated diffusion (GLUT-1 to GLUT-5). GLUT-4 is the main protein that promotes glucose transport into skeletal muscle cells and adipocytes. When insulin receptors are not properly phosphorylated, the signal stimulating GLUT-4 transport activity is not created, and glucose capture by cells is consequently reduced while stimulation of insulin production remains continuous, resulting in IR. Figure 1 shows a flow chart of the main mechanisms of NLFD development.

NAFLD molecular mechanisms

Studies have suggested the involvement of several genes associated with free acid metabolism, oxidative stress reduction, xenobiotic metabolism, and fibrogenesis in the physiopathogenesis of NASH. A thorough literature review on the multiple roles of peroxisome proliferator-activated receptors (PPARs) at the cell and tissue levels showed that the association of NAFLD with lipid and carbohydrate metabolism and inflammation involves PPAR activation. These receptors represent a subgroup of transcription factors activated by ligands belonging to the hormone nuclear receptor family that are differentially expressed in the liver, adipose and muscular tissue, PPARα, PPARγ and PPARβ/δ, respectively.

Overexpression of liver PPARα controls lipid catabolism and is the target of hypolipidemic drugs. PPARγ regulates adipocyte differentiation and lipid storage and is the target of thiazolidinediones, which are drugs used in insulin sensitization indicated in type 2
diabetes treatment. Activation of PPARβ/δ increases lipid catabolism in skeletal muscles and in heart and adipose tissue, helps prevent weight gain, and suppresses macrophage-derived inflammation.34

PPARs are also expressed in dendritic cells, macrophages, and B and T lymphocytes, which suggests a role in immunity by shifting the Th1/Th2 equilibrium towards the Th2 anti-inflammatory response. PPARα is also expressed in endothelial cells, where it regulates the expression of leukocyte adherence molecules. Activation of PPARα promotes the regulation of several inflammatory response components such as chemokines and cytokines, decreases expression of Th1 T-bet transcription factor (expressed by T cells), and increases GATA-3 (guanosine adenosine timidine adenosine 3) expression, a well-known positive regulator of Th2 cytokines with anti-inflammatory properties. PPAR agonists may inhibit nuclear factor-κB (NF-κB) transcription activity, which mediates the expression of genes responsible for inflammation, suggesting a possible therapeutic effect of PPAR ligands in the treatment of inflammatory diseases such as NAFLD.34

Steroid regulator element binding protein 1 (SREBP-1C) is another transcription factor member of the SREBP family that also plays a crucial role in cellular lipid metabolism. SREBP proteins activate the synthesis of cholesterol, fatty acids, and phospholipids in the liver parenchyma. The SREBP-C isoform participates in liver fatty acid and triglyceride synthesis by stimulating the synthesis of enzymes important for lipogenesis such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS).35

Lipogenic enzymes may also be stimulated by transcription factors such as carbohydrate responsive element-binding protein (ChREBP). ChREBP plays a crucial role in lipogenesis by regulating the transcription of lipogenic genes, including ACC and FAS.36 An in vivo study showed that ChREBP regulates lipogenesis in vivo and plays a defining role in the development of liver steatosis and IR in mice.37

There is also evidence that some nutrients can induce or attenuate the activation of the aforementioned transcription factors (SREBPs, ChREBP, and PPARs) and thus interfere in the expression of genes related to carbohydrate and lipid metabolism and inflammation related to NAFLD pathogenesis.38-40 This interaction between nutrients and genes is the focus of nutrigenomics, a relatively recent field that investigates the effects of ingested foods, their nutrients, and bioactive compounds on gene expression and biological processes that might affect human health.41,42

Given the previous discussion regarding the possible mechanisms involved in NAFLD, it is important to ask how we can act on modifiable risk factors to prevent or treat NAFLD, keeping in mind that NAFLD interacts with other chronic diseases. Therefore, dietary habits are a relevant issue in the treatment of patients with NAFLD.

**Soy-mediated mechanisms in preventing and treating NAFLD**

Although hormone-mediated body metabolism regulation, activation of transcription factors, inflam-
mation, and lipid metabolic pathways are rated the central axes in NAFLD development, food intake and physical inactivity are also relevant factors in the physiopathology of this disease. Previous reviews on diets that reduce NAFLD recommend foods rich in polyunsaturated fat (ω-3 fatty acids), fruits, vegetables, low-glycemic-index foods, and foods with high-fiber content for patients with NFLD.43,44

The quality of dietary protein is also a matter of discussion, and recent studies have shown the benefits of plant protein in preventing and treating non-transmissible chronic diseases. Studies have shown that soy-mediated mechanisms that reduce lipogenesis might involve interactions between soy protein, the isoflavonoid-enriched fraction, and amino acids pattern. These soy components modulate lipid and carbohydrate metabolism in the liver through the expression of related transcription factors.45-48

The data observed in experimental models show that soy and its components are able to interfere with NAFLD physiopathology mechanisms.

**Lipid metabolism**

One of the possible effects of soy is related to its ability to stimulate PPARα, which would likely increase fat oxidation in the liver and may minimize liver steatosis. Soy polyunsaturated free acids and amino acid patterns are believed to activate PPARα, thus promoting transcriptional regulation of several genes known as the PPARα transcriptome, resulting in increased mitochondrial and peroxisomal β-oxidation.36,42,43

An experimental study showed that rats fed soy protein exhibited a significant increase in PPARα-regulated gene expression compared to casein-fed animals. These findings indicate that soy modifies the patterns of gene expression in the liver, which might contribute to a decrease in lipid accumulation in liver tissue.40,44,45 An analysis of gene expression in animal model revealed that soy protein isolate (SPI) significantly reduced liver steatosis by activating the PPARα nuclear receptor, which suggests that soy may be useful in managing nonalcoholic liver diseases.50

Another feature that soy might influence is the inhibition of SREBP-1. Soy protein is able to decrease liver lipogenesis via molecular mechanisms involving inactivation of the SREBP transcription factor and consequent suppression of target genes involved in liver fat synthesis.40,51

Another experimental study showed that rats fed soy protein exhibited significantly lower SREBP-1 expression than casein-fed rats, thus suggesting that soy protein affects liver lipid synthesis through gene transcription. Soy protein intake also decreased fatty acid synthesis and the expression of the malic enzyme, resulting in a decrease in lipid, triglyceride, and cholesterol storage in the liver tissue, whereas casein-fed animals exhibited a higher rate of liver steatosis.52

Modulation of gene expression via SREBP-1 was also observed in an experimental model of induced obesity. Rats fed soy-based diets exhibited decreased expression of SREBP-1 and increased expression of SREBP-2, a transcription factor responsible for reducing cholesterol synthesis and absorption in the liver,
compared to casein-fed rats. These results show that the type of protein consumed can modulate lipid metabolism in the adipose and liver tissues even in the presence of dietary fat via SREBP transcription factors.

### Insulin resistance

Clinical trials and animal models have shown that soy protein intake aid in the maintenance of normal glucose and insulin serum levels. Therefore, experimental models are being developed to elucidate the possible soy-dependent mechanisms involved in carbohydrate metabolism and NAFLD pathophysiology.

The hyperglycemic clamp is considered the gold standard for assessing the in vivo functional ability of the pancreas β-cells. Experiments using the hyperglycemic clamp demonstrated that a soy protein diet decreased the stimulation of insulin release independent of the presence of isoflavones. Additional tests using euglycemic-hyperinsulinemic clamps, considered the gold standard for assessing IR in vivo, showed that soy protein attenuated IR in spite of a high-fat diet. In another experimental model, administration of different protein types (soy versus casein) combined with dietary fat showed that soy decreased PPARγ expression in the pancreas, which decreased the synthesis of GLUT-2 mRNA, thus decreasing glucose transport into the pancreas and minimizing insulin release. Low insulin release might by itself minimize peripheral IR and help in maintaining normal glycemia.

Soy and its components might also increase peripheral insulin sensitivity by reducing oxidative stress and/or modulating pro-inflammatory cytokines. Pro-inflammatory cytokines can inhibit the insulin signaling cascade in peripheral tissues. Gudbrandsen et al. observed that obese rats with liver steatosis that consumed isoflavonoid-enriched soy protein supplements exhibited a decrease in pro-inflammatory cytokine (TNF-α and IL1) serum levels.

Because cytokines are partly produced by oxidative stress, the antioxidant activity of soy could also promote a decrease in pro-inflammatory cytokines and, consequently, in IR. A recent study investigated the effects of soy protein on liver steatosis, NASH, and IR. Animals were assigned to 4 different groups; 2 groups were given NASH-inducing diets with and without soy protein, and 2 were given standard diets with and without soy protein. The results showed additional effects of soy on the antioxidant system by increased superoxide dismutase and catalase activity and decreased cytochrome P450 2E1 protein expression compared to the standard diet groups. The authors concluded that soy protein might improve the liver function of NASH patients by attenuating IR and reducing free radical formation.

### Action of soy isoflavonoids

The action of isoflavonoids on NAFLD is still not clear; they may function as bioactive compounds that influence the modulation of genes that promote oxidation and inhibit the synthesis of fat, as antioxidants that attenuate oxidative stress, or as modulators of the inflammatory process.

Studies performed with obese rats with liver steatosis fed isoflavonoid-enriched soy protein supplements reported positive effects on liver inflammation biomarkers. Reduction of serum levels of aspartate transaminase (AST), ALT, and pro-inflammatory cytokines such as TNF-α and IL-1 as well as liver protection against oxidative damage were observed in these animals. Based on these results, the authors suggested that isoflavonoid-enriched soy protein might be useful functional food for treating steatohepatitis in clinical practice.

Similar results were observed in a study analyzing the protective effect of soy isoflavonoids in an induced-NASH experimental model; the tissue and serum levels of malondialdehyde, which is the main marker of oxidative stress, were significantly lower in the isoflavonoid group. Moreover, the isoflavonoid group exhibited increased activity of the antioxidative enzyme paraoxonase, whereas the levels of total cholesterol and triglycerides were reduced and steatosis, inflammation, necrosis, and fibrosis were attenuated. Therefore, it seems that soy-derived isoflavonoids are effective in preventing hepatocellular damage and reducing lipid peroxidation in an induced-NASH model.

The beneficial effect of soy extract with several doses of isoflavonoids on glucose tolerance and liver function was shown by a diabetes experimental model. In animals that were given 3.0 mg of isoflavonoids, glycemia levels during fasting and after oral glucose administration were significantly lower than in the control group, which was not given phytoestrogen. The results showed that isoflavonoid-supplemented soy extract could be beneficial for diabetic animals by inducing weight loss, improving glucose tolerance, and protecting against hepatocellular damage.

The effect of specific isoflavonoids has awakened the interest of the scientific community. A study performed with young adult non-obese rats to assess the isolated action of genistein (GEN) applied 3 different diet types: casein with or without GEN and SPI with GEN. Animals fed the SPI diet exhibited significant reductions in body weight, abdominal fat, and liver lipid and triglyceride levels compared to animals fed casein with or without GEN. Thus, reduction of body weight and fat does not seem to involve GEN. However, the authors did not rule out the possibility of GEN being involved in other mechanisms that might contribute to liver steatosis.

To compare the physiological effects of a diet containing soy protein on the liver metabolism of fatty
Acids, rats were fed casein- or soy-based isonitrogenous diets with or without soy-derived isoflavonoid supplements at various doses (1.06 or 8.51 g/kg). Diets containing soy protein without isoflavonoids reduced the activity and expression of enzymes involved in the liver synthesis of fatty acids, particularly SREBP-1. However, the addition of isoflavonoids increased the synthesis of PPARα mRNA in a dose-dependent manner. These results suggest that soy protein rather than isoflavonoids is the primary nutrient responsible for the activity of soy in decreasing liver lipogenesis. Moreover, soy protein supplements may increase the expression of electron-uncoupling proteins (UCPs) in adipose tissue through a PPARγ-dependent mechanism, an effect that is associated with the anti-obesity activity of soy.\(^7\)

Rats fed soy with small amounts of isoflavonoids exhibited greater expression of UCP-1 and thus gained less weight than casein-fed animals, partly due to an increase in the thermogenic ability mediated by UCP-1. Moreover, the area of fat droplets in brown adipose tissue was significantly lower in animals fed soy-based diets than in casein-fed rats.\(^8\)

A further effect of soy and its isoflavonoids concerns the inhibition of the transcription factor liver X receptor (LXR). The activity of LXR might inhibit the activation of SREBP-1c, which modulates the expression of enzymes involved in fatty acid synthesis. Rats fed SPI with or without isoflavonoids exhibited increased PPARα expression and decreased LXR expression. These findings might partially explain the anti-steatosis effects of soy by means of improving lipid homeostasis and insulin sensitivity.\(^9\)

**Conclusion**

Despite abundant scientific evidence from experimental models showing the protective effect of soy in NAFLD, it is not yet well established whether this effect is related to the amino acid profile, polyunsaturated fatty acids, or the isoflavonoids in this food.

Because previous clinical trials have shown that soy intake decreases the serum levels of lipids, reduces IR, and maintains glycemia homeostasis, the use of this food might yield positive effects in preventing and/or treating NAFLD by reducing steatosis in patients with NASH. This effect may be attributed to the attenuation of mechanisms involved in NAFLD pathogenesis such as reducing lipid levels in the serum and liver and attenuating IR, oxidative stress, and inflammation. However, we stress the need for clinical trials to confirm the specific effect of soy as a therapeutic agent in NAFLD.

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