5. Phytosterols in type 2 diabetes and obesity – Molecular mechanisms of action

Małgorzata Zakłos-Szyda

Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 4/10 St., 90-924 Lodz, Poland

Abstract. Epidemiological data indicate that type 2 diabetes (T2D), which is often associated with overweight or obesity, is one of the main health problems of the world. T2D disease is characterized mainly by hyperglycemia (increased level of blood glucose concentration after meals), irregularities in the insulin secretion or attenuated insulin sensitivity and disturbed lipid metabolism (high cholesterol levels). It has been proven that some of the plant lipids present in human diet, such as sterols and stanols, can effectively reduce the level of cholesterol by competing with dietary and biliary cholesterol during intestinal absorption. Recently some of studies identified phytosterols as one of the key modulators of glucose metabolism, which could lead through the AMP-activated kinase (AMPK) activation or peroxisome proliferator-activated receptors (PPARs) to transcriptional regulation pathways. The aim of this review is to present phytosterols as regulators of carbohydrates and lipids metabolism as well as molecular mechanisms of their activities and antidiabetic properties.

Correspondence/Reprint request: Dr. Małgorzata Zakłos-Szyda, Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 4/10 St., 90-924 Lodz, Poland. E-mail: malgorzata.zaklos-szyda@p.lodz.pl
Abbreviations

ACC - acetyl-CoA carboxylase; AMPK- AMP-activated kinase; ChREBP - cholesterol regulatory element binding proteins; FAS - fatty acid synthase; HDL - high-density lipoprotein; HNF-4α - hepatic nuclear factor 4α; LXR- liver X receptor; LDL- low-density lipoprotein; LDLR - LDL receptor; LXRE- LXR response element; PPARs- peroxisome proliferator-activated receptors; PPRE, PPAR response element; RXR - retinoid X receptor; SREBP- sterol regulatory element binding proteins; T2D - type 2 diabetes; CVD – cardiovascular disease; VLDL - Very low density lipoprotein; PS -plant sterol, phytosterol; TAG–triglycerides

Introduction

Epidemiological data indicate that type 2 diabetes (T2D) and obesity are one of the main and the most expensive chronic diseases in the world. According to The International Diabetes Federation data (2012) about 8.3% of world population suffers from diabetes and it has been estimated that in 2030 this value will exceed 10% [1]. These estimations include millions of undetected cases, because at the beginning the disease is often free of symptoms and is only diagnosed years later. Type 2 diabetes, which is usually a result of wrong dietary habits and reduced physical activity, represents 85-95% of all diabetes cases and among other diet related diseases is the major cause of deaths. The disease is characterized mainly by hyperglycemia (increased level of blood glucose concentration after meals), irregularities in the insulin secretion or attenuated insulin sensitivity and disturbed lipid metabolism. The most dangerous complications of T2D are hypertension, hypercoagulability, retinopathy, nephropathy, atherosclerotic cardiovascular diseases and innervation of the lower limbs (known as diabetic foot) [2,3]. There is evidence that more than 60% of patients with 2TD suffer hepatic steatosis associated with elevated level of serum triglycerides (TAG), low density lipoprotein (LDL) and upregulated cholesterol synthesis accompanying insulin resistance [4, 5]. What is more, there is an increasing tendency of T2D occurrence not only among adult population, but also among children.

Current therapies for dyslipidaemia in T2D and obesity include agents that lower LDL cholesterol level primarily through increasing hepatic LDL-receptor activity or cholesterol absorption inhibition, sterol-regulatory element binding protein cleavage-activating protein (SCAP), activating ligands of PPAR-alpha/gamma/delta, liver X receptor (LXR) agonists and finally activators of AMP-activated kinase.
Phytosterols in type 2 diabetes and obesity – molecular mechanisms of action

The long-term drug therapies very often are limited due to their side-effects or lack of patient’s response to the specific drug. It is well established that in addition to genetic predisposition diet significantly lowers the risk and symptoms of T2D, cardiovascular disease or obesity [6, 7]. Dietary fats besides fatty acids contain approx. 5% of other fat-soluble chemicals called non-glyceride components [8]. Results of epidemiologic studies confirm that some of these lipid components, especially phytosterols, can effectively reduce by 10% the level of LDL cholesterol or glucose in the blood [9, 10]. The importance of deliberately composed diet as prevention agent confirms the fact that the long term exposure to lower LDL cholesterol beginning early in life has been found to reduce the risk of cardiovascular diseases by 55%, compared with a statin treatment started later in the life [11]. The aim of this review is to present phytosterols as regulators of carbohydrates and lipids metabolism as well as molecular mechanisms of their activities and antidiabetic properties.

Chemical structure, dietary sources and intakes of phytosterols

Phytosterols (plant sterols) are structural and functional analogues of animal major sterol – cholesterol. They are synthesized only by plants [12] and exist as integral components of plant cell membrane, mitochondria outer membrane and the endoplasmic reticulum, which are associated with glycosphingolipids, such as glucosyl ceramide, in raft-like subdomains. Analogically to cholesterol in animal cells they regulate membrane permeability, signal transduction events, activity of membrane-bound enzymes and decrease membrane fluidity. They belong to polyisoprenoid compounds which possess carbon skeletons structurally derived from polymerization of isoprene unit (CH$_2$=C(CH$_3$)CH=CH$_2$) [13]. Similar to cholesterol they are C28 or C29 carbon steroid alcohols with one hydroxyl group; their structural difference relates to containing more of methyl or ethyl groups and one or two double bonds in the side chain [Table 1] [14]. Phytosterols can be distinguished in to three main groups: 1) sterols (also called Δ5-sterols, with double bond between the C5 and C6 atoms); 2) Δ7-sterols (with a double bond between the C7 and C8 atoms, rarely occur in nature); 3) stanols (molecule without double bond, thus reduced sterols) [14].

In nature they may exist as free compounds or as esters of fatty acids, hydroxycinnamic acid, glucose or glycolipids, although estrified forms mixture may represent 50% of the total sterol content [15]. Since phytosterols are sparingly soluble in lipids and insoluble in water they are added to food as esters with significantly increased solubility [16].
Table 1. The structures of cholesterol, typical plant sterols and stanols and Aloe vera phytosterols [12, 20].

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical structure</th>
<th>Chemical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>cholest-5en-3(\beta)-ol</td>
</tr>
<tr>
<td>Campesterol</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>24-methylcholesta-5en-3(\beta)-ol</td>
</tr>
<tr>
<td>(\beta)-Sitosterol</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>24-ethylcholesta-5en-3(\beta)-ol</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>24-methylcholesta-5,22-dien-3(\beta)-ol</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>24-ethylcholesta-5,22-dien-3(\beta)-ol</td>
</tr>
<tr>
<td>(\beta)-Sitostanol</td>
<td><img src="image6" alt="Chemical structure" /></td>
<td>3(\beta),5(\alpha)-stigmasstan-3-ol</td>
</tr>
<tr>
<td>Campestanol</td>
<td><img src="image7" alt="Chemical structure" /></td>
<td>24-methyl-5(\alpha)-cholestan-3(\beta)-ol</td>
</tr>
<tr>
<td>Awenasterol</td>
<td><img src="image8" alt="Chemical structure" /></td>
<td>24-ethylcholesta-5,24(28)-dien-3(\beta)-ol</td>
</tr>
<tr>
<td>Lophenol</td>
<td><img src="image9" alt="Chemical structure" /></td>
<td>4-methylcholest-7-en-3-ol</td>
</tr>
<tr>
<td>Cycloartanol</td>
<td><img src="image10" alt="Chemical structure" /></td>
<td>9,19-cyclolanostan-3-ol</td>
</tr>
</tbody>
</table>

So far there have been identified more than 200 phytosterols among which the most common are \(\beta\)-sitosterol (24-\(\alpha\)-ethylcholesterol) (90%), campesterol (24-\(\alpha\)-methylcholesterol) and stigmasterol (\(\Delta\)22, 24-\(\alpha\)-
ethylcholesterol). Although all plant tissues contain phytosterols their richest source are oils extracted from plant seeds, namely 952 mg of phytosterols/100 g are present in corn oil, while the edible corn seeds contain only 70 mg/100 g [17]. The main source of plant sterols in human nutrition are rapeseed oil, soybean oil, sesame seeds, wheat germ, nuts (walnuts, peanuts, hazelnuts), almonds, and legume seeds (Table 2) [17, 18]. Usually they content mixture of different phytosterols, however the occurrence of some is limited to the one plant family, for example brassicasterol (present in rapeseed oil) is synthesized by Brassicaceae (Table 3) [17].

It is believed that the minimum amount of phytosterols having a hypocholesterolemic effect is one gram per day, however daily doses beyond 2 – 3 g do not show additional reductions [21, 22, 23]. Some data indicate that people’s diets provide very small amount of plant stanols (20 - 50 mg) and sterols (150 - 400 mg) per day [16, 24]. These amounts are not sufficient to reduce the level of blood cholesterol by competing of their absorption. Additionally the majority of phytosterols consumed is effectively excreted from the body and only 0.5 – 2 % of sterols and 0.04- 0.2 % of stanols are absorbed (by comparison, intestinal absorption of cholesterol is 45 - 54 % of intake). It is known that β-sitosterol is secreted into the bile, stored in the gallbladder and after incorporated into feces subsequently [25, 26].

Recently the interest in phytosterols in human diet has not been related only to their activity of lowering blood cholesterol levels, but also to their hypoglycemic properties.

**Table 2.** Phytosterols content in selected food products [17, 18, 20 with modification].

<table>
<thead>
<tr>
<th>Source of sterols</th>
<th>Content of sterols [mg/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice brans</td>
<td>1190</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>221</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>865</td>
</tr>
<tr>
<td>Olive oil</td>
<td>176</td>
</tr>
<tr>
<td>Rapeseed oils</td>
<td>663-881</td>
</tr>
<tr>
<td>Corn oil</td>
<td>952</td>
</tr>
<tr>
<td>Palm oil</td>
<td>49</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>725</td>
</tr>
<tr>
<td>Almonds</td>
<td>143</td>
</tr>
<tr>
<td>Beans</td>
<td>76</td>
</tr>
<tr>
<td>Corn</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 3. Content of phytosterols in selected plant oils [20 with modification].

<table>
<thead>
<tr>
<th></th>
<th>Rapeseed oils</th>
<th>Soybean oil</th>
<th>Sunflower oil</th>
<th>Corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total content of phytosterols [mg/100 g]</td>
<td>633 – 881</td>
<td>460</td>
<td>410</td>
<td>970</td>
</tr>
<tr>
<td>Total content of esterified phytosterols [mg/100g]</td>
<td>398 – 435</td>
<td>57,6</td>
<td>207</td>
<td>565</td>
</tr>
<tr>
<td>Cholesterol [%]</td>
<td>0,1 – 0,4</td>
<td>0,3</td>
<td>0,1</td>
<td>0,1</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>10,8 – 16,2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Campesterol</td>
<td>27,6 – 34,4</td>
<td>18,1</td>
<td>7,5</td>
<td>17,2</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0,1 – 0,8</td>
<td>15,2</td>
<td>7,5</td>
<td>6,3</td>
</tr>
<tr>
<td>Δ7-stigmasterol</td>
<td>2,1 – 2,3</td>
<td>1,4</td>
<td>7,1</td>
<td>1,8</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>48,7 – 52,3</td>
<td>54,1</td>
<td>58,2</td>
<td>60,3</td>
</tr>
<tr>
<td>Awenasterol</td>
<td>0,1 – 2,1</td>
<td>2,5</td>
<td>4,0</td>
<td>10,5</td>
</tr>
<tr>
<td>Δ7-awenasterol</td>
<td>0,8 – 1,9</td>
<td>2,0</td>
<td>4,0</td>
<td>1,1</td>
</tr>
</tbody>
</table>

Phytosterols and AMPK

One of the first studies indicating hypoglycemic properties of phytosterols demonstrated that in hyperglycemic rats the glucose level was lowered and the insulin level was increased after oral administration of β-sitosterol [27]. However, the molecular mechanisms underlying these beneficial effects of phytosterols had been largely unknown. Recent findings have revealed that the increasing of glucose uptake induced by β-sitosterol is mediated by adenine monophosphate-activated protein kinase, known as an AMP-kinase (AMPK) [28]. The involvement of that specific protein was discovered during research conducted on chemically pure compounds used in the treatment of type 2 diabetes, such as metformin (biguanide derivative of Galega officinalis L.) or adenosine analogue AICAR (5-aminoimidazole riboside-4-carboxamide). Therapeutic effect of these anti-diabetes medications is related to lowering of cellular ATP levels and thereby increasing of AMP concentration [29]. That process leads to change in the cellular energy state, which is manifested in the increased AMP:ATP ratio and therefore reduction of available energy amount. The increase of AMP level is a factor activating the AMP-activated protein kinase. As the result of AMP-kinase activation in hepatocytes one can observe reduction of glucose production, inhibition of fatty acid synthesis and activation of catabolic pathways, such as fatty acids oxidation or glycolysis.

The AMPK protein is a serine-threonine kinase that is present in almost all mammalian tissues [30]. It is a heterotrimer composed of a catalytic α and
Phytosterols in type 2 diabetes and obesity – molecular mechanisms of action

regulatory β and γ subunits. Change of intracellular energy status or increased intracellular Ca\(^{2+}\) level leads to the allosteric binding of AMP to the γ subunit which is accompanied by phosphorylation of the Thr-172 residue located in catalytic domain by upstream AMPK kinases: liver kinase B1 (LKB1) or calcium/calmodulin-dependent protein kinase kinase (CaMKK) [31]. After its activation AMPK phosphorylates downstream substrates to switch off ATP-consuming pathways. Due to the fact that AMP-kinase is involved in the processes of ATP generation and its consumption limitation, the most common models used in the in vitro studies of AMPK signaling pathways are muscle cells (myocytes), liver (hepatocytes) and fat cells (adipocytes) [31]. In muscle cells the active enzyme stimulates glucose uptake in the insulin-independent manner by increasing the transcription of the gene encoding the membrane GLUT4 glucose transporter or increases the ability to capture sugar molecule by GLUT1 transporter protein. Taking into account liver cells activation of AMPK decreases blood glucose levels in insulin-independent manner by inhibition of gene expression of proteins involved in the process of gluconeogenesis: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P). It is known that expression of these genes is controlled by variety of transcription factors, such as CREB protein (cAMP-response element-binding protein), HNF4-α (hepatocyte nuclear factor- 4α) or FOXO (forkhead transcription factor O) and activated AMPK is their negative regulator [30, 32, 33].

In type 2 diabetes increased glucose production is associated with inability of insulin to suppress hepatic gluconeogenesis and promote glycolysis. Insulin inhibits gluconeogenesis through modulation of FOXO (which possess a sterol sensing domain). In mammals four isoforms of the FOXO family (FOXO1, FOXO3, FOXO4 and FOXO6) have been identified so far. It is known that through FOXO phosphorylation AMPK controls transcription of gluconeogenic genes, i.e. phosphoenolpyruvate C kinase and glucose-6-phosphatase [34, 35]. Moreover, it is known that induction of lipogenesis in response to insulin is critically dependent on the transcription factor - sterol regulatory element-binding protein-1c (SREBP-1c). In vivo studies showed that constitutively active FOXO1 reduced basal expression of SREBP-1c mRNA in mice liver by \(\sim 60\%\) [36]. In addition, one of the genes controlled by FOXO factors is \(p27\) gene encoding a protein of the same name, which is an inhibitor of the cell cycle. It has been shown that the AMPK through \(p27\) protein phosphorylation inhibits cycle progression under circumstances of imbalanced energy [37].

NHF-4α protein is a transcription factor regulating the expression of liver specific genes. That protein, like PPARs, is known as “sensor” nuclear
receptor responding to incoming dietary signals and metabolites generated in the organism [38]. It occurs most commonly in the liver, where it is involved in the process of differentiation of hepatocytes, but is also present in the intestine, kidney and pancreatic β cells [39]. It belongs to the family of co-receptors of steroid and thyroid hormones. It binds to specific DNA sequences as a dimer and regulates expression of PEPCK.

AMP-kinase mediates lipid metabolism through several lipid metabolism-related transcription factors, such as peroxisome proliferator activated receptors (PPARs), sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP), which promote the conversion of glucose to fatty acids [40]. SREBP-1c and ChREBP regulate enzymes involved in fatty acid synthesis, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). Direct phosphorylation of acetyl-CoA carboxylase Ser-79 by AMPK leads to ACC inactivation and therefore decreased fat synthesis through inhibition of acetyl-CoA to malonyl-CoA conversion. Activation of AMPK also increases the phosphorylation of hydroxymethylglutaryl-CoA reductase (HMG-CoA) resulting in reduced cholesterol synthesis by inactivation of HMG-CoA reductase.

SREBP is a family of DNA binding proteins that act as the primary transcription factors in regulating the synthesis of fatty acids. There are known two mammalian forms of the protein: SREBP1 and SREBP2. The SREBP-1c isoform gene expression is regulated by insulin - glucagon blocks the effect of insulin – and involved in synthesis of free fatty acids and triglycerides (e.g. ACC and FAS enzymes), while the SREBP-2 controls cholesterol homeostasis through expression of genes encoding proteins such as low density lipoprotein receptor (LDLR) or HMG-CoA reductase [41]. Both transcription factors are synthesized as precursors and lowering cholesterol level induces their release into cytoplasm. As mature SREBP molecules they combine into dimers and translocate into the nucleus, where they bind to DNA within sterol regulatory elements (SRE) [42]. The SREBP-1c gene is also potently activated by liver X receptors (LXR) - nuclear receptors that are sensors of cholesterol metabolism and lipid biosynthesis. LXR form heterodimers with other superfamily of nuclear receptors - retinoid X receptors (RXR), which are known to provide structural and signaling support to its heterodimerizing partner (also for PPARs). LXR promote binding of LXR–RXR dimers to LXR-responsive elements (LXREs) and thus improve glucose tolerance in a mouse model of diet-induced obesity [43]. It has been shown that 5-campestenone reduced
level of SREBP-1c [46], and the same observation was obtained after treating of ZDF rats (Zucker diabetic fatty rats, an animal model of T2D) with aloe vera phytosterols (lophenol and cycloartanol) [19].

Phosphorylation and activation of AMPK is regulated also by other biological active substances, like adiponectin. Adiponectin is an adipocyte-derived peptide from white adipose tissue, which has anti-inflammatory and insulin-sensitizing properties. Several studies in humans and other mammals have shown that low circulating levels of adiponectin is associated with obesity, type 2 diabetes and metabolic syndrome [44]. It has been shown that adiponectin plays a crucial role in hepatic lipid metabolism because it suppresses the transcriptional activity of SREBP-1c via the complex containing AdipoR1 (one of the two functional receptors for adiponectin)/LKB1/AMPK [45], thus it promotes lipid oxidation, suppresses lipid synthesis and reduces hepatic lipid accumulation.

Studies performed by Hwang et al. have indicated that β-sitosterol (20µM) treatment of L6 muscle cells increases glucose uptake through increased translocation of GLUT4 to the plasma membrane and decreases intracellular triglycerides and cholesterol content by stimulation of LKB1-mediated AMPK activation [28]. In that case β-sitosterol significantly increased phosphorylation of the Thr-172 AMPK α subunit and Ser-79 ACC. After three weeks of dietary exposure of ZDF rats to 5-campestenone (0.6 %) it was observed reduction of plasma total cholesterol, limited rise of blood glucose levels after oral administration of high glucose concentration, increased insulin response and reduced visceral fat mass in rats [46].

It was shown β-sitosterol dose-dependently stimulated glucose uptake (0,1 – 100 µM) in primary rat preadipocytes and subsequently differentiated adipocytes and induced lipolysis in adipocytes [47].

Majority of studies identifying antidiabetic activity of phytosterols focuses on Aloe vera extracts, which are used in manufacture of food and drink products, pharmaceuticals, and cosmetics. So far there have been identified at least five different anti-hyperglycemic phytosterols from Aloe Vera gel which are derivatives of lophenol and cycloartanol (Table 1) [19]. Administration of 1 µg of Aloe vera phytosterols to type 2 diabetic BKS.Cg-m/Leprdb/J (db/db) mice lowered blood glucose levels, however did not reduce the serum concentrations of cholesterol. It was observed that the effective dose that was applicable to decrease serum cholesterol levels was 100-fold higher than that one applicable to decrease blood glucose levels. On the other hand it is suggested that the mechanism of phytosterols activity may be connected to different structures of the compounds studied: anti-hyperglycemic phytosterols derived from Aloe vera gel are 4-monomethyl and 4-dimethyl sterols, while effective structure for the
reduction of serum cholesterol is the 4-desmethylmoiety (containing no methyl groups at carbon atom 4). Analysis of the effects after oral administration of lophenol and cycloartanol (25 µg/(kg day) daily for 44 days) in Zucker diabetic fatty (ZDF) rats has confirmed that these compounds can reduced blood glucose, serum FFA, and TG levels and visceral fat accumulation. Recently it has been shown that Aloe vera phytosterols work as weak agonists of PPARα and PPARγ [48].

**Peroxisome proliferator-activated receptors (PPARs)**

Studies carried out with chemically pure synthetic compounds—thiazolidinediones—revealed that the key mechanism of insulin resistance and hyperlipidemia pharmacological treatment targets peroxisome-proliferator-activated receptors (PPARs) activation [49]. These compounds are currently available and widely used in T2D therapy. Unfortunately they can have undesirable side effects (i.e. weight gain, fluid retention and heart failure), so there is a need of search for better and less toxic agents from plants, fruits and vegetables.

PPARs are members of the nuclear receptor superfamily which act as transcription factors for genes linked to lipid metabolism, glucose homeostasis, inflammation and vascular physiology. They bind to the PPAR response element (PPRE) of target genes as a PPAR/retinoid X receptor (RXR) heterodimer. Their natural ligands are polyunsaturated fatty acids such as arachidonic acid, linoleic acid, eicosanoids, phospholipids, lysophosphatidic acid and their metabolic products [50]. Since PPAR-regulated genes are involved in the adaptation of cells or organs to metabolic changes, as the in vitro cellular models for their activity research are used myocytes (skeletal muscle), hepatocytes (liver) and adipocytes (fat cells).

There have been identified three mammalian PPAR isoforms: PPAR-α, PPAR-β/δ and PPAR-γ, each with a different ligands affinity, target genes and biological role [51, 52]. The expression of PPAR isoforms varies widely from tissue-to-tissue. PPAR-α is expressed in many cells that have active fatty acid oxidation capacity, including heart, liver, brown adipose tissue, muscles and epidermis - it reduces plasma triglyceride level, increases transport of HDL particles from peripheral tissues to the liver and is involved in regulation of energy homeostasis [53]. After short-term starvation of PPARα null-mice there was observed hepatic steatosis, myocardial lipid accumulation and hypoglycemia [54]. Synthetic ligands of PPAR-α – fibrates (mainly gemfibrozil, fenofibrate and clofibrate) are a class of hypolipidemic agents used in the treatment of hypertriglyceridemia [55]. Ikeda et al. showed that PPARα activation by campestenone suppressed
expression of SREBP1c mRNA through the decrease of LXR/RXR formation [46, 56]. They observed that in rats fed with oxidized derivative of campesterol (campest-5-en-3-one) drastically increased the activities and the mRNA levels of lipolytic enzymes in the liver, which was accompanied by reduction of the activities and mRNA expressions of lipogenic enzymes such as ACC, FAS, glucose-6-phosphate dehydrogenase and pyruvate kinase. What is more, Jones and coworkers observed that consumption of dietary phytosterols reduced plasma and hepatic triglycerides in mice and that was accompanied by reduction in mRNA expression of intestinal PPARα, which is a key mediator of intestinal bile acid transport [57]. In vitro studies have shown that exposure of HepG2 cells to phytosterols limited cellular lipid availability and reduced secretion of apolipoprotein B100 (apoB100) and circulating TAG concentration [58, 59]. It is known that apoB100 is a component of liver major secretory product—VLDL, which serves as transporter of endogenously synthesized lipids to peripheral tissues [60]. On the other hand Graf and coworkers did not observe a decrease in triglycerides secretion in sterol transporter G5G8 knock-out mice (which opposes the absorption of plant sterols) challenged with a plant sterol-free low fat or high fat diet nor did they observe reductions in mRNAs for PPARα and its target genes or rates of fatty acid oxidation in primary hepatocytes. That shows that phytosterol accumulation does not regulate the development of fatty liver disease or loss of glycemic control directly [61].

PPAR-β/δ is the predominant isoform in skeletal muscle, small intestine and liver, and its activation enhances fatty acid oxidation and increases exercise endurance capacity. It has been shown that transgenic mice with PPAR-β/δ activation in adipose tissue were resistant to high-fat diet-induced and genetically predisposed obesity and hyperlipidemia, while PPAR-β/δ knockout mice showed reduced energy uncoupling and were prone to obesity under high-fat diet feeding [62]. What is more, PPARβ/δ overexpression attenuates insulin resistance in obesity and enhances insulin action and glucose tolerance [51].

Activation of PPARγ, expressed predominantly in adipose tissue, immune system cells and to a lesser extent in liver, promotes β-oxidation of fatty acids, causes insulin sensitization and enhances glucose metabolism [63]. Its significance in the organism is directly proved: the homozygous PPARγ gene deletion is lethal during the first days of mice embryonic development [64]. There are three distinct protein forms arised by differential transcription start sites and alternative splicing: PPARγ1 (expressed in most tissues), PPAR γ2 (present predominantly in adipose tissue) and PPARγ3 (high expressed in macrophages, white adipose tissue and large intestine) [52]. The PPARγ activation is essential for the
adipocyte differentiation process (it is known as “master regulator of adipogenesis”), because it enhances expression of adiponectin which is essential for adipocytes differentiation. Since promotion of adipocyte differentiation results in increase of the number of insulin sensitive cells and adiponectin secreting small adipocytes, thus ligands for PPARγ may be novel candidates for diabetes treatment due to their positive influence on systemic insulin sensitivity [65]. Recent report has indicated that aloe vera phytosterols (lophenol and cycloartanol) acted as ligands for both PPAR α and γ present in livers of mice with diet-induced obesity [48].

Additionally, activation of PPARγ leading to significant reduction of derived from obese adipose tissue proinflammatory adipocytokines, such as TNFα (tumor necrosis factor-α), interleukin-6 (IL-6) and MCP-1 (monocyte chemoattractant protein-1), was found as the result of three weeks supplementation of hyperlipidemic individuals with 2 g plant sterols per day [66, 67]. It has been suggested that the anti-inflammatory activity of PPARγ may involve interference with proinflammatory transcription factors (like NF-κB) or prevention of removal of corepressor complexes from gene promoter regions leading to reduction of inflammatory genes transcription [68].

There are some data indicating that activation of PPARγ receptor can also lead to insulin-independent reduction of blood glucose levels by inhibiting expression of genes encoding proteins involved in gluconeogenesis, especially PEPCK and glucose-6-phosphatase [69]. Studies performed with β-sitosteryl and campesteryl derivatives from rice bran have shown their activity to up-regulate blood adiponectin levels via indirect activation of PPARγ through NF-κB inhibition [70]. It is known that ATP-binding cassette half-transporters complex G5G8 opposes the absorption of dietary sterols and promotes the excretion of cholesterol into bile. Resent study has shown, that hypertriglyceridemia in G5G8-deficient mice induced by phytosterols was associated with increased hepatic PPARγ expression level and several of its target genes [61].

Summarizing, phytosterols effectively decrease gluconeogenesis and lipogenesis processes (Figure 1). That activity predominantly appears to be mediated by AMPK and PPAR receptors and is accompanied mainly by inhibition of PEPCK and G6P genes expression or inhibition of ACC and FAS enzymatic activities. However, in order to identify in details their effect on the specific enzymes and their regulators (transcription factors) involved in glucose and lipid metabolism (like glycolysis, gluconeogenesis, glycogenolysis, lipogenesis) further investigations should be performed.
Phytosterols in type 2 diabetes and obesity – molecular mechanisms of action

**Figure 1.** Effects of phytosterols on glucose and lipid metabolism.

**Conclusion**

Phytosterols are recommended as dietary modifiers of serum lipids and, therefore, various PS-enriched foods and supplements are available on the market [71]. The effect of lowering plasma cholesterol concentrations by phytosterols in normal and moderately hypercholesterolemic patients have been known since about 1950 [71]. Some phytosterols could reduce visceral fat accumulation and would be useful for the improvement of hyperglycemia. However, recently obtained data suggest, that therapy using increased sitosterol and campesterol concentrations has been shown to increase risk of premature coronary atherosclerosis in individuals homozygous for phytosterolemia, also known as sitosterolemia [72]. It is a rare autosomal-recessively inherited disease resulting from mutations in either the ABCG5 or ABCG8 genes coding adenosine triphosphate-binding cassette transporters, ABCG5 and ABCG8, which limit PS absorption from the intestine and facilitate PS excretion via bile. Contrary to normally poorly absorbed diet-delivered phytosterols (5%) in sitosterolemic patients sitosterol absorption can reach to 60%, which increases plasma PS concentrations and deposition of sterols in skin and tendons (xanthomas) and in the walls of the coronary arteries [73]. Additionally, there is growing evidence that in parenteral nutrition plant sterols may contribute to intestinal
failure-associated liver disease [74, 75, 76]. Because phytosterols may cause different biological effects, thus the molecular mechanisms of their activities should be thoroughly examined and new strategies involving their use in prevention and treatment of type 2 diabetes mellitus should be validated through large-scale population trials.

Acknowledgments

This work by the author was supported in part by grants from NCN (Grants No. 3407/B/P01/2011/40, UMO-2011/01/B/NZ9/04699) and NCBiR(Grant No. PBS1/B8/7/2013).

References


55. Varga T., Czimmerer Z., Nagy L. (2011) PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation Biochim Biophys Acta 1812, 1007–1022.


