Cholesterol Metabolism and Its Regulation by Functional Foods

Chim-Chi Yasser¹, Betancur-Ancona David², Jimenez-Martinez Cristian¹, Chel-Guerrero Luis², Davila-Ortiz Gloria¹

¹Departamento de Ingeniería Bioquímica, Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional. Avenida Wilfrido Massieu S/N, Col. Industrial Vallejo. ZP. 07738. Ciudad de México, México.
²Facultad de Ingeniería Química, Campus de Ciencias Exactas e Ingenierías. Universidad Autónoma de Yucatán. Periférico Norte, KM 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn, ZP. 97203. Mérida, Yucatán, México.

Abstract—Currently, obesity is considered an epidemic due to the disruptions it causes to health, highlighting the incensement in cardiovascular diseases associated with cholesterol and low-density lipoprotein (LDL) high concentrations. However, cholesterol is also involved in various metabolic and structural functions vital to human biology. This homeostasis can be modified by external factors such as medications or by internal factors such as diseases or metabolic changes generated by the type of diet at which each person is exposed. In this sense, the research points to the knowledge of functional foods, which provide beneficial health effects and prevent the risk of disease. It has been reported that hypocholesterolemic type bioactive peptides obtained by enzymatic hydrolysis of various seeds such as soybeans, rice and sunflower. A similar effect is observed with unsaturated fatty acids, which have antithrombotic and antiarrhythmic effects, prevent atherosclerosis, contribute to decrease blood pressure and reduce the concentration of triglycerides, total cholesterol and lipoproteins of very low-density lipoprotein (VLDL) in plasma. Therefore, these compounds incorporated in foods are considered functional, since its bioactive potential could be used to prevent cardiovascular disease.

Keywords—cholesterol, functional foods, hypocholesterolemic.

I. INTRODUCTION

Obesity is a chronic disease originated by various causes and with numerous complications, characterized by body fat excess that threatens the health of the individual. Its growth is considered a risk worldwide, an epidemic. In 2013, the obesity in men was 36.9% and 38% in women¹. In 2030, the number of obesity people will increase at 573 million². Also, obesity carries high concentrations of total cholesterol and of the one found in the low-density lipoproteins (LDL), which are strongly associated with a risk increment in cardiovascular diseases, for this reason a reduction in total cholesterol and LDL in hypercholesterolemic individuals reduces the incidence of these diseases³.
liver and they distribute triglycerides and cholesterol to peripheral cells, where they become LDL after partial depletion has occurred in triglycerides, due to vascular LPL activity. LDL are considered “bad” cholesterol because it causes the release of cholesterol in peripheral tissues. In contrast, high-density lipoprotein (HDL) contains A1 and A2 apolipoproteins (APOA1 and APO2, respectively), which serve as acceptors and trap cholesterol from peripheral tissues effectively. VLDL are the primary cholesterol source for the peripheral tissues via the LDLr, which is the major regulatory step to adjust the importance of this biomolecule. Export from cells requires both the expression of ABC superfamily (belt conveyors coupled to ATP) and extracellular presence of apolipoproteins as free cholesterol acceptors. If cholesterol is in excess, the body accelerates its conversion into bile acids, allowing its elimination in the stool, which is the only route of excretion of this substance. On the other hand, if the supplement of cholesterol is low, the de novo synthesis is carried out in the liver.

De novo synthesis requires that genes involved in cholesterol production be transcribed, such as LDLr and HMGR. These genes are transcribed in terms of the amount of sterols detected by some cellular transcription factors. The cholesterol reservation obtained from de novo synthesis by hepatocytes will subsequently be esterified by ACAT and incorporated into VLDL APO B-100, which will be secreted into blood and transported into tissues. Also, the peripheral tissues contribute to hepatic cholesterol reservation through its transfer to the liver in a process mediated by HDL. Cellular transport of this substance can change LDLr synthesis, which contributes to cell and blood cholesterol concentration. Hormones, such as estrogen, thyroid hormone and insulin, modulate this process. During aging, and mRNA increased LDLr, with decreasing exposure to cholesterol contributes to hypercholesterolemia and cardiovascular disorders.

When the human body is subjected to a diet high in cholesterol, serum and liver cholesterol concentration increases and lipoproteins (VLDL and LDL) concentration increases too, which is considered a cardiovascular risk factor. Several studies have shown that the high cholesterol concentration in serum and liver, such as triglyceride, from diets rich in cholesterol, have a direct relationship between the amount administered and the period of the diet. However, some authors report an opposite effect on serum triglyceride levels. Hu y col, found that a 1% cholesterol diet in rats show a decrease of more than 50% of the serum triglyceride levels, but increases the hepatic levels of them, also the serum and liver figures of the molecule are maintained. Other studies made, in laboratory animals, have tried also to establish the metabolic behavior of these diets: rats fed high cholesterol diets have an increment in serum and hepatic cholesterol concentration. Rats show an increment in serum and hepatic triglyceride levels, which conditions a non-alcoholic liver disease (NAFLD). Given this evidence, it can be established a relationship between dietary cholesterol and triglyceride metabolism, which is evident mainly in the liver tissue. However, lipid homeostasis in vertebrates is mainly regulated by a family of membrane-bound transcription factors known as sterol regulatory element binding proteins (SREBP).

2.1 Sterol regulatory element binding proteins
The SREBP are transcription factors with three regions: i) A N-terminal fragment, which is actually a family b/HLH/LZ (basic/helix-loop-helix/leucine zipper) transcription factor with a tyrosine residue in the basic region of the b/HLH motive that allows it to join to the SRE sequences of the erythrocyte membrane; ii) a central domain containing two transmembrane regions separated by 31 amino acids located in the endoplasmic reticulum; and iii) a regulatory carboxyterminal domain. SREBP has 3 isoforms: SREBP-1a and 1c and SREBP-2. These proteins regulate the expression of over 30 genes involved in the metabolism and intake of cholesterol, fatty acids, triglycerides and phospholipids and in the reduced nicotinamide adeninedinucleotide (NADPH) metabolism, which is required for the synthesis of these molecules.

The SREBP-1 and SREBP-2 proteins share 47% homology. The SREBP-1a and 1c transcripts are produced to be used as an alternative start site of transcription and differ in the first exon (exon 1a and 1c). SREBP-1a is a more potent transcriptional activator that SREBP-1c due to its NH2-terminal higher transactivation domain. However, SREBP-1c isoform is predominantly expressed in most human and mice tissues, with high levels particularly in the liver, white adipose tissue, skeletal muscle, adrenal glands and brain. In contrast SREBP-1a is highly expressed in cell lines and tissues with high capacity for cell proliferation, such as the spleen and intestine. SREBP-1a is considered a potent activator of all SREBP-responsive genes, including those that mediate the cholesterol, fatty acids and triglycerides synthesis. SREBP1c preferentially power the genes required for fatty acids synthesis and SREBP-2 has a large transcription domain has, but it preferentially activates cholesterol synthesis (Fig. 1).

At cellular level, to monitor the sterols level in the erythrocyte membrane, the cell uses two proteins: The SREBP cut activating enzyme (SCAP) and the HMG-CoAR. These proteins share an intramembranal sequence called Sterol Screening Domain (SSD). Through this domain, sterols cause that SCAP and HMG-CoAR bind to
amembrane protein of the erythrocyte, which is an insulin inductor (INSIG). The INSIG protein produces a crossroad between the transcriptional and post-transcriptional regulatory mechanisms that ensure cholesterol metabolism\cite{11}. In presence of this molecule, the SREBP are retained in the erythrocyte; in its absence they are released by proteolysis that allows the activation of target genes that control lipid metabolism\cite{12}. Molecularly it occurs as follows: after the translation of the mRNA, SREBP precursors are retained in the erythrocyte membrane through an association with the SCAP. Under low cholesterol conditions, the SCAP accompanies the SREBP precursors from the erythrocyte to the Golgi apparatus where two functionally different proteases, site 1 of protease (S1P) and site 2 of protease (S2P), hydrolyzed sequentially the precursor protein releasing nuclear SREBP (nSREBP) in the cytoplasm\cite{12}. In contrast, when cells have abundant cholesterol SCAP binds to INSIGN, stabilizing the protein and allowing the accumulation of a stable complex INSIG/SCAP/SREBP. In consequence, the content of SREBP and INSIGN decrease, serving as a reservoir for SREBP. When cells are lacking cholesterol, SCAP/SREBP of INSIGN dissociates and then it is degraded in proteasomes. The free SCAP/SREBP complex binds proteins from the rough endoplasmic reticulum and migrates to the Golgi apparatus where SREBP is processed into nSREBP. This activates the transactivation genes of the cholesterol biosynthetic enzymes and LDLR. At the same time, nSREBP activates genes for INSIGN, for this reason it relates to the carbohydrates metabolism\cite{13,14}.

There are three factors that selectively regulate the SREBP-1c transcription: the liver X activated receptor (LXR), insulin and glucagon. Studies in animals fed with a high cholesterol diet and the use of oxysterols synthetic agonists have shown that LXRα and LXRβ nuclear receptors form heterodimers with the retinoid X receptor (RXR), which are activated by a variety of sterols including oxysterolintermediaries and produce an expression of lipogenic genes and high speed of lipogenesis\cite{15}. Thus, LXR functions as a sensor for cholesterol levels and promotes its excretion and clearance. Therefore, when a rich cholesterol diet is consumed, SREBP-1c is activated by LXR to induce oleate synthesis, which participates in the synthesis of cholesterol esters, required for transport and storage\cite{16}. Also, another function of the liver is to convert carbohydrates excess to fatty acids for storage as triglycerides. Insulin stimulates the synthesis of fatty acids in response to carbohydrates excess. SREBP-1c mediates the lipogenic effect of insulin in the liver. Insulin increases the mRNA levels of SREBP-1c and regulates the expression of their target genes. In liver, both SREBP-1c and SREBP-2 transcription is stimulated by the SREBP by a feedback mechanism that requires sterol regulatory elements (SRE) sequences in the promoters of these genes\cite{17}. The SREBP-1c expression to normal levels, promotes the biosynthetic pathway of fatty acids, while SREBP-2 promotes the synthesis of cholesterol. Genes who respond to the SREBP-1c activation include ATP citrate lyase (which produces acetyl CoA), the acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)\cite{13}. FAS protein is a cytosolic protein and a major lipogenic enzyme in mammals. It catalyzes the reactions that contribute to the conversion of acetyl-CoA andmalonyl-CoA to palmitate (C16:0). FAS gene transcription is under strict nutritional and hormonal control in lipogenic tissues strict, such as the liver and adipose tissue\cite{16}. Other SREBP-1c target genes encode fatty acids elongation complex limiting enzymes, which
increasing glucagon levels. This relationship is reversed
The SREBP-1c total amount in liver and adipose tissue is
reduced during fasting, by decreasing insulin levels and
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the function of this gene to generate a predisposition to
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phosphogluconate dehydrogenase gene, required to
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III. FUNCTIONAL FOODS
Functional foods are defined as specific food substances
that promote health as part of a varied diet. In general,
they provide beneficial effects in contributing to the
maintenance of health status and reducing the risk of
illness. They are natural or processed foods that, in
addition to its nutritional components, contain additional
components, nutritious or not, to help maintain or
improve health and promote physical fitness and mental
state of the person who consumes them. There are
flavonoids that neutralize free radicals focusing on lower
the risk to develop cancer; carotenoids that contribute to
eye health; fiber that reduces the risk of colon cancer and
various bioactive peptides with antioxidant, antimicrobial
and antihypertensive effects, among others. Some of the
components forming part of functional food are shown in
TABLE 1. However, there are specific components such
as hypocholesterolemic unsaturated fatty acids and
bioactive peptides capable of reducing the levels of
triglycerides and cholesterol
convert palmitate into stearate (C18:0); the stearoyl CoA
desaturase, which converts stearate into oleate (C18:1) and
the glycerol 3-phosphate acyltransferase, enzyme that participates in the synthesis of phospholipids and triglycerides. Also, SREBP-1c and SREBP-2 activate three genes: the malic enzyme (ME) gene, glucose 6 phosphate dehydrogenase (G6PD) gene and 6-phosphogluconate dehydrogenase gene, required to generate NADPH, which is consumed throughout the lipids biosynthetic pathway. Malic enzyme presents in cytosolic (ME1) and mitochondrial (ME2) form. The ME1 catalyzes reversibly the oxidative decarboxylation of malate to pyruvate, carbon dioxide and NADPH, and then contributes to the de novo synthesis of fatty acids via
FAS. It has been found that mutations in ME eliminate
the function of this gene to generate a predisposition to obesity and diabetes type 2.
The SREBP-1c total amount in liver and adipose tissue is
reduced during fasting, by decreasing insulin levels and
increasing glucagon levels. This relationship is reversed
during feeding. SREBP-1c overexpression in the liver of
transgenic mice produces a rich in triglycerides fatty liver
without increasing cholesterol, whereas SREBP-2 over
expression in transgenic mice results in a 28% increment
in cholesterol synthesis. In rat hepatocytes, insulin
injections increase the total amount of SREBP-1c. SREBP-1a overexpression in mouse liver markedly increases the expression of genes involved in cholesterol
synthesis (such as HMGCoA, HMGCoAR, squalenesynthetase) and fatty acid synthesis (such as
ACC, and FAS) causing the accumulation of such
molecules. Studies in rats using orotic acid, a compound
known for the ability to produce nonalcoholic fatty liver
disease (NAFDL) showed an increment in de novo
lipogenesis from elevated FAS, ME and G6PDH activity
in rat liver. Other studies shown that the activity of FAS,
G6PDH and MEIn the liver is increased in presence of
hepatic steatosis. Even Tang y col, established that
inhibition of the SREBP pathway can be used as a
therapeutic strategy for treating diseases of lipid
metabolism including type II diabetes and
atherosclerosis.
As it was mentioned before, cholesterol metabolism is
under strict genetic regulation and different routes are
used for absorption, synthesis and secretion. Knowing the
metabolic pathways activated during the intake of high
cholesterol diets opens strategies that could potentially
counteract the effects caused by this type of food. Another
protein called Peroxisome Proliferation Activated
Receptor (PPAR) is also involved in the regulation
expressed in hepatocytes and cardiomyocytes. This
protein is required for the fatty acids to express its
function in the genetic processes and participates in a
large network of genes that regulate the metabolism of
lipids and glucose, and the differentiation of adipocytes.
There are several types of PPAR: α, β and γ. PPAR α is associated with fatty acid metabolism in the liver, kidney, heart, skeletal muscle and brown adipose tissue. PPAR γ
is associated more with other adipose tissue. Some
medications such as fibrates and thiazolidinediones act by
activation of PPAR. The effects of these receptors in
metabolism range from peroxisomal proliferation,
increment in fatty acid oxidation, reduction in plasma
triglyceride levels, and improvement in glucose tolerance.
It is important to remember that the fatty acids are energy
elements of the body, and they modulate its metabolism,
synthesis and oxidation through an allosteric enzyme
action. In this way, omega fatty acids regulate lipogenic,
mitochondrial oxidative and gluconeogenic enzymes.
Long chain polyunsaturated fatty acids reduce hepatic
lipogenesis and consequently reduce the amount of
enzymes involved in lipid synthesis; they also modulate
adipogenesis. Another regulatory mechanism that refers
to triglycerides adipose tissue is the storage of a wide
range of fatty acids, which differ in their molecular
structure. The output of fatty acids from adipose tissue of
a subject is selective; it dependson the size of the chain
and the degree of unsaturation. This has been observed in
vitro in human and animal adipocytes.
As remarkable progress, genes related to the different
metabolic pathways of lipids provide insight into the
effects and responses to various diets, which generates
changes in health from the nutritional status of the
individual. Therefore, there will be a beneficial effect to
consume diets that include functional foods and their
bioactive components.
antiarrhythmic effects, increase bleeding time preventing

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omega 6

group at one end and a methyl group at the other. The

hydrocarbon straight chains terminating in a carboxyl

fatty acids. From the chemical standpoint, fatty acids are

unsaturated. Aldo, may be classified in monoun saturated

and polyunsaturated. b) For their length of the chain they

can be classified as short (4-6 carbons), medium (8-12

carbons), long (14-18 carbons) or very long (20 or more

carbons) chain.According to the position of the first

double bond in the chain, called omega, counting from the

methyl end, there are three families of polyunsaturated

fatty acids: ω-3, ω-6 and ω-9. Some are classified as

essential because the human body cannot synthesize them

and they are needed for vital functions, these are the ω-3

and ω-6 families commonly known as omega 3 and omega 6.

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to lower blood pressure and reduce the concentration of
plasma triglyceride, and they decrease the total

cholesterol and VLDL. In the nervous system, unsaturated fatty acids are necessary for proper
development and functioning of the brain and nervous
system. They are concentrated in the retina and cerebral
cortex, and have the ability to correct visual and brain
problems in patients with deficiencies. Unsaturated fatty
are hormonal precursors compounds also, such as
prostaglandins and thromboxanes, which facilitate the
transmission of messages in the central nervous system.

Unsaturated fatty are precursors of other fatty acids such as
archidonic, eicosapentaenoic (EPA) and
docosahexaenoic acid (DHA). DHA is part of the
membranes of retinal photoreceptors.

It is also important to remember that all cell membranes
contain lipid bilayers and are impermeable to charged
molecules, for communication between cells and
compartments, which require from protein transporters or
receptors that are embedded in this double layer, occur.
Furthermore, a flow mechanism that causes the lateral
movement of proteins and invagination, that allows
endocytosis and exocytosis, is observed. This fluidity
requires long chain unsaturated fatty acids, because
saturated fatty acids decrease this vital characteristic,
even more when proteins have to collide with other
molecules in various biochemical processes. For example,
the role of insulin to communicate its signal during
glucose metabolism in rats is damaged when food
provides more than 10% of dietary calories from saturated
fatty acids, which can be improved with the intake of
omega-3 fatty acids.

It has been shown that the antiarrhythmic effect of omega
fatty acids may be associated with functional balance
between the vague and sympathetic systems whose
modulation is involved with the cardiac response. In
particular EPA and DHA could compare its effect with
some medications used in the treatment of these cardiac
problems, which has been observed when fish oil is
supplied to rats in treatment, due to adrenoceptor involved
in arrhythmias. These receptors are membrane proteins
that transmit the catecholamines neuroendocrine message
in the pace and strength of cardiac contraction. In this
process DHA has similar activity to the one presented by
the β-blocking molecules. In relation to atherosclerosis, it
is the product of a long period of artery inflammation and
endothelial dysfunction plays an important role on it,
because the flow associated with this function is mediated
by nitric oxide and aggravated by atheroma. Omega fatty
acids provide more fluidity to membranes of endothelial

| Component | Food | Potential benefit |
|-----------|------|-------------------|
| Flavonoids (Catechins and flavonols) | Green tea and citrus | Neutralize free radicals and reduce risk of cancer |
| Carotenoids (β-carotene and lutein) | Carrots and green vegetables | Improve vision |
| Fiber | Shell grains | Reduces the risk of colon cancer |
| Unsaturated fatty acids (ω-3 y ω-6) | Fish oils and some grasses | Reduce levels of cholesterol and triglyceride |
| Bioactive peptides (antioxidants, ACE inhibitors, hypocholesterolemic) | Eggs, meat, chickpea, soy, milk, rice, sunflower, and others. | Reduce the risk of cardiovascular disease and degenerative |

Table 1: Chemical components with functional potential present in many foods.
cells promoting the synthesis or output of nitric oxide. To prevent atheroma, it is suggested the intake of omega fatty acids from fish sources. It has been observed in neutrophils and monocytes that fish oil reduces the formation of oxygen-derived free radicals and increased nitric oxide production in cultured human endothelial cells, which is beneficial to avoid atheromas. As it has been already mentioned, another factor associated with heart problems is the concentration of HDL-cholesterol that may be a predictive factor to avoid the risk of coronary heart disease. Although, the current recommendations for the treatment of dyslipidemia do not include specific HDL-cholesterol values, the acceptable range was 35 mg/dL and it has been modified to 40 mg/dL. Statins, which are 3-hydroxy-3-methyl-CoA reductase inhibitory molecules, are drugs used for reducing cholesterol and increase the HDL at moderate levels. Other resources for this purpose are, omega fatty acids, which are obtained from the oil of various animals and plants. Vegetable fats and oils are usually obtained from seeds or the outer layer of the fruit. The percentage of this oil reserves widely vary from about 5% in cereals to 68% in coconut. In plants and therefore in vegetable oils, factors such as the type of crop, agricultural land and climatic conditions have a strong influence on the content of fatty acids. Mediterranean populations consume large quantities of olive oil (rich in oleic acid, which helps in the formation of ω-9 acids), vegetables and fish; it was found that consumption of fish and olive oil over the life of these populations might provide independent protective effect on the development of many diseases. Currently, there is interest in the oil obtained from Chia seed (Salvia hispanica), due to its high content of unsaturated fatty acids.

3.2. Bioactive peptides
Currently the study of dietary proteins as functional and beneficial components has received much attention because the generation of bioactive peptides is under investigation. The term bioactive is used to describe components with various types of biological activity, such as antimicrobial, immunomodulatory, regulating intestinal transit, antioxidants, ACE inhibitory, hypocholesterolemic and antithrombotic. Bioactive peptides are defined as amino acid sequences of between 2 and 20 residues without activity in the original protein and presenting biological activity when released by hydrolysis. They cross the intestinal epithelium and reach peripheral tissues via systemic circulation, exerting specific functions locally, gastrointestinal and systemic. These peptides may alter cellular metabolism and present these functions. Its activity is similar to a hormone or drug that modulates a physiological role through its interaction with a specific receptor triggering a physiological response. Typically, the bioactive peptides are hydrophobic and absorbed between 70 and 80% faster compared to free amino acids.

The method for obtaining these molecules is by the hydrolysis of proteins; this is accomplished by chemical processes (acids or bases) or by biological processes (using enzymes). Biological processes are the most recommended if the products will be used in the food field. According to Guadix y col, the proteases or proteolytic enzymes are commercial grade enzyme mixtures, liquid or solid and are classified in various ways (TABLE 2).

However, it should be considered the temperature, pH, hydrolysis time and degree of hydrolysis (DH) to obtain hydrolyzed with specific characteristics, such as the proper distribution of molecular masses of the peptides formed, the amino acids released, and the amount of residual undigested protein. The temperature is selected to optimize the kinetics of the enzyme or mixture of them, using a range from 32 to 50 °C. The pH is determined according to the range where the enzyme activity is at its highest. The hydrolysis time is related in direct proportion with the DH. This is a measure of the proteins hydrolytic degradation ability. It is defined as the percentage of broken peptide bonds in relation to the total of them in the original protein. DH is considered practical and convenient to control the hydrolytic processes and as the largest indicator used for the comparison of different protein hydrolysates. In response to DH, hydrolysates can be classified as: a) Limited hydrolysates (DH <10%). They are used to improve the functional and technological properties, because an increase in solubility occurs. It also improves the emulsifying power, foaming and absorption of water or oil, which can be used in baked goods and mayonnaise. b) Extensive hydrolysates (DH ≥ 10%). Used in specialized feeding, either as a protein supplement or medical diets. These hydrolysates include the ones that seek to exploit or improve the nutritional characteristics of the protein source, because the peptides obtained have higher thermal stability and reduced allergenicity. Its size can be more effectively absorbed in the gastrointestinal tract as compared to the intact proteins. Its high solubility allows their use in liquid foods.
Table.2: Classification of proteases according to source, action and catalytic site.

| Classification | Diversity | Description |
|----------------|-----------|-------------|
| Source         | Animals   | They are extracted from animal tissues, plants, bacteria or fungus and its metabolites or its metabolites |
|                | Vegetables| Hydrolyze peptide bonds along the protein chain. |
|                | Bacterial | Hydrolyzed amino terminals (aminopeptidase) or carboxyterminal (carboxypeptidase). |
|                | Fungal    | Endoprotease able to hydrolyze amino acid linked to specific substrates |
| Catalytic action | Endoprotease | Endoprotease able to hydrolyze amino acid linked to specific substrates |
|                | Exopeptidase | Exopeptidase able to hydrolyze amino, carboxyl or both |

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Regarding the hypocholesterolemic effect, it has been reported more incidence of this activity in plant sources compared to animals. Specifically, the case of soybeans mentioned, both recommended by the Food and Drug Administration (FDA) and the American Heart Association (AHA), because by consuming 25 g/day of this protein as part of a diet low in saturated fat there is a decrease in cholesterol levels. An estimated consumption amount of protein decrease lipoprotein LDL up to 8% in patients with high cholesterol levels, while it has no adverse effects in people with normal cholesterol levels. By reducing serum cholesterol and LDL concentration there is a positive effect as the atherogenic index (LDL/HDL) decreases in rats, mice and humans. However, when taken orally these proteins are target of gastrointestinal proteases, which originate bioactive peptides and contribute to the effects mentioned before. It has been reported that soy peptides minimize serum cholesterol levels compared to the intact protein. Compared with casein, these hydrolysates also reduce serum cholesterol because the excretion is promoted through feces, because it cannot be absorbed. Similarly, it decreases the oxidation of LDL and triglyceride levels and enhance the vascular activity. One possible explanation for these events is given by the reduced levels of hepatic lipogenic enzymes such as G6PDH and stimulation of adiponectin, a hormone involved in adipocyte differentiation, and insulin and fatty acids sensitivity. Based on these facts, Nagaokay col found that cholesrlomicellar solubility was significantly lower during the intake compared to hydrolyze soy protein. The same result was observed in serum, liver and intestines of rats, indicating inhibition of cholesterol absorption due to the inability of the micelles to solubilize the molecule. It has also been found the hypocholesterolemic effect in glycinn, specifically in the Leu-Pro-Tyr-Pro-Argpentapeptide, obtained from soy protein, as mentioned.
before. This pentapeptide reduced serum cholesterol in mice when administered orally in doses of 50 mg/kg and has a homologous enterostatin (Val-Pro-Asp-Pro-Arg) structure\textsuperscript{58}. It has been shown that sunflower hydrolysates generated when using pepsin or alcalaseproteases and undergo to intestinal digestion in vitro, inhibit cholesterol incorporation into the micellar suspensions of bile salts. A similar effect was obtained with rice bran hydrolysates, which managed to reduce total serum cholesterol and increase HDL in male Wistar rats\textsuperscript{59}.

Concerning triglycerides, Ascencio y col reported that consumption of soy protein isolates maintained at a low level liver stores of these esters\textsuperscript{60}. It is also reported that the isolated protein maintains in a low level plasma triglycerides, increases adiponectin, accelerates lipid metabolism and decreases the body fat of obese rats and mice\textsuperscript{61,62}.

IV. CONCLUSIONS

Lipids we eat in the diet are important for the growth and development of human beings, both for its energy function, and for the supply of essential fatty acids. However, consumption of saturated fatty acids is harmful to the body, as it promotes the formation of cholesterol associated with low-density lipoproteins (LDL) and increases the rigidity of cell membranes, compromising their biological functions. Therefore, controlled fat intake may be beneficial to health, to prevent certain cardiovascular diseases. Unsaturated fatty acids protect at cardiovascular level, because they modulate serum cholesterol levels and reduce susceptibility to oxidation of LDL. The \( \omega-3 \) and \( \omega-6 \) acids prevent chronic inflammation, some carcinogenic processes and degenerative diseases, because they are precursors of eicosanoids or affect the transcription of some genes involved in the development of these diseases. These \( \omega-3 \) and \( \omega-6 \) acids exist in foods such as cold-water fish. But their presence has been reported in various vegetables like amaranth and olive, although chia has stood out due to its high content of these acids. It is known that plant foods provide some metabolites with different biological properties, being the hypocholesterolemic activity, due to enzyme peptides origin, one that has received attention in recent years. When these peptides are ingested as part of the diet they have a role in reducing the risk of cardiovascular diseases, which have been guaranteed by in vitro and in vivo studies.

However, knowledge of how these ingredients work on the body is not sufficiently consolidated. This is due to the complexity of the multiple interactions between the constituents of the food during the digestive process and the impact on the metabolism of the same. The different lifestyles, age, health status and dietary habits among populations, even within the same society, make it difficult to generalize the results of studies and indicate that a bioactive ingredient is not necessarily effective for all consumers. Therefore, there are still many aspects of food/health relationship at different stages of life and to individuals in different metabolic situations that require research. For this it is necessary to demonstrate the biological activity of each specific food, this by performing clinical studies and evaluating the potential health risks of these functional foods to meet the risk-benefit balance that their consumption entails for the population.

REFERENCES

[1] Vandevijvere, S., Chow, C., Hall, K., Umali, E., Swinburn, B. “Increased food energy supply as a major driver of the obesity epidemic: a global analysis”. Bull World Health Organ. 93, 2015. pp:446-456.

[2] Kelly, T., Yang, W., Chen, C., Reynolds, K., He, J. “Global burden of obesity in 2005 and projections to 2030”. International Journal of Obesity. 32, 2008. pp: 1431-1437.

[3] Ye, D., Lammers, B., Zhao, Y., Meurs, I., Van Berkel, T., Van Eck, M. “ATP-Binding Cassette Transporters A1 and G1, HDL Metabolism Cholesterol Efflux, and Inflammation: Important Targets for the Treatment of Atherosclerosis”. CurrentDrug Targets. 12 (5), 2011. pp:647-660.

[4] Sanhueza, J., Valenzuela, R., Valenzuela, A. “El metabolismo del colesterol: cada vez más complejo”. Grasas y aceites. 63 (4), 2011. pp:373-382.

[5] Navarro, V., Zabala, A., Gómez, S., Portillo, M. “Metabolismo del colesterol: bases actualizadas”. Revista Española de Obesidad; 7, (6), 2009. pp:360-384.

[6] Meurs, I., Van Eck, M., Van Berkel, T. “High-density lipoprotein: key molecule in cholesterol efflux and the prevention of atherosclerosis”. Curr. Pharm. Des. 16, 2010;pp: 1445-1467.

[7] Davis, H., Zhu, L., Hoos, L., Tetzloff, G., Maguire, M., Liu, J. “Niemann-pick C1 like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole body cholesterol homeostasis”. The Journal of Biological Chemistry, 279(32), 2004. pp:33586-33592.

[8] Hossain, Z., Sugawara, T., Aida, K., Hirata, T. “Effect of dietary glucosylceramide from sea cucumber on plasma and liver lipids in cholesterol-fed mice”. Fisheries Science, 77, 2011. pp: 1081-1085.
[9] Trapani, L., Segatto, M., Pallottini, V. “Regulation and deregulation of cholesterol homeostasis: the liver as a metabolic "power station"”. Journal Hepatology, 4(6), 2012. pp:184-190.

[10] Martini C. “Cholesterol: from feeding to gen regulation”. Genes Nutr; 2, 2007. pp: 181-193.

[11] Kim, A., Chiu, A., Barone, M., Avino, D., Wang, F., Coleeman, C., Phung, O. “Green tea catechins decrease total and low-density lipoprotein cholesterol: a systematic review and meta-analysis”. J. Am. Diet Assoc. 111, 2011. pp:1720-1729.

[12] Okuhira, K., Fitzgerald, M., Tamehiro, N., Ohoka, N., Suzuki, K., Sawada, J., Naito, M., Nishimaki-Mogami, T. “Binding of PDZ-RhoGEF to ATP-binding cassette transporter A1 (ABCA1) induces cholesterol efflux through RhoA activation and prevention of transporter degradation”. J. Biol. Chem. 285, 2010, pp:16369-16377.

[13] Hu, X., Wang, Y., Wang, F., Xue, Y., Li, Z., Nagao, K., Yamagita, T., Xue, Chang, H. “Dietary saponins of sea cucumber alleviate orotic acid induced fatty liver in rats via PPARa and SREBP 1c signaling”. Lipids in Health and Disease, 9, 2010. pp:25.

[14] Takahashi, Y., Soejima, Y., Fucusato, T. “Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis”. J Gastroenterol, 18, 2012: pp:2300-2308.

[15] Najafi-Shoushtari, S., Kristo, F., Li, Y., Simmen, F., Al-Dwarii, A., Pabona, J., Simmen, R., Simmen, F. “Cytoplasmic malic enzyme 1 (ME1) mediates high fat diet-induced adiposity, endocrine profile, and gastrointestinal tract proliferation-associated biomarkers in male mice”. PLOS ONE, 7(10): 2012. e46716.

[16] Ahmed, M., Byrne, C. “Modulation of sterol regulatory element binding proteins (SREBPs) as potential treatments for non-alcoholic fatty liver disease (NAFLD)”. Drug Discovery; 12(17), 2007. pp:740-747.

[17] Sato R. “Sterol metabolism and SREBP activation”. Arch. Biochem. Biophys. 501, 2010. pp:177-181.

[18] Espenshade, P. “SREBPS: sterol-regulated transcription factors”. Cell Science, 119, 2006. pp: 973-6.

[19] Ronis, M., Chen, Y., Badeaux, J., Badger, T. “Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling”. J. Nutr. 139, 2009. pp:1431-1438.

[20] Althunibat, O., Hashim, R., Taher, M., Daud, J., Ikeda, M., Zaimuddin, B. “In vitro antioxidant and antiproliferative activities of three Malasia sea cucumber species”. Eur J Sci Res, 37 (3), 2009. pp: 376-387.

[21] Horton, J., Goldstein, J., Brown, M. “SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver”. J Clin Invest, 109, 2002. pp: 1125-1131.

[22] Jeon, B., Kim, Y., Choi, W., Koh, D., Kim, M., Yoon, J., Kim, M., Hur, B., Paik, P., Hur, M. “Increases FAS expression by modulating the DNA binding of SREBP-1c and Sp1 at the proximal promoter”. J Lipid Res, 53(4), 2012. pp: 755-66.

[23] Zhao, C. and Dahlman-Wright, K. “Liver X receptor in cholesterol metabolism”. Journal of Endocrinology, 204, 2010. pp: 233–240.

[24] Wu, J., Wan, C., Li, S., Li, S., Wang, W., Li, J. “Thrp promotes hepatic lipogenesis, and its expression is regulated by LCRa thorough and SREBP1c-dependent mechanism”. Hepatology, 10, 2012. pp:10-12.

[25] Al-Dwarii, A., Pabona, J., Simmen, R., Simmen, F. “Cytoplasmic malic enzyme 1 (ME1) mediates high fat diet-induced adiposity, endocrine profile, and gastrointestinal tract proliferation-associated biomarkers in male mice”. PLOS ONE, 7(10): 2012. e46716.

[26] Zhang, B., Xue, C., Hu, X., Xu, J., Li, Z., Wang, J. “Dietary sea cucumber cerebroside alleviates orotic acid-induced excess hepatic adipopexis in rats”. Lipids in Health and Diseases; 11, 2012. pp: 48.

[27] Zhang, L., Reue, K., Fong, L., Young, S., Tontonoz, P. “Feedback regulation of cholesterol uptake by the LXr-IDOL-LDLR axis”. Arterioscler Thromb Vasc Biol, 2012. pp:32.

[28] Tang, J., Li, J., Qi, W., Qiu, W., Li, P., Li, B., Song, B. “Inhibition of SREBP by a small molecule, betulin, improves hyperlipidemia and insulin resistance and reduces atherosclerotic plaques”. Cell Metab; 13,(1), 2011. pp:44-56.

[29] Burri, L., Berge, K., Wirbrand, K., Berge, R., Bargel, J. “Differential effects of krill oil and fish oil on the hepatic transcriptome in mice”. Front Genet. 2, 2005. pp:45.

[30] Choi, J., Bae, J., Kim, D., Li, J., Kim, J., Lee, J., Kang, H. “Dietary compound quercitrin dampens VEGF induction and PPARgamma activation in oxidized LDL-exposed murine macrophages: association with scavenger receptor”. CD36. 58, 2003. pp: 1333-1341.

[31] Kussmann, M., Bladeren, P. “The extended nutrigenomics – understanding the interplay between the genomes of food, gut microbes, and human host”. Frontiers in genetics, 2(21), 2011. pp: 1-13.

[32] Mataix, J. “Nutrición y alimentación humana”. Nutrientes y alimentos. Océano/ergon, 1, 2002. pp:421.
[33] Vioque, J., Pedroche, J., Yust, M., Lquiri, H., Megías, C., Girón, J., Alazí, M., y Millán, F. “Péptidos bioactivos en proteínas vegetales de reserva”. Brazilian Journal of Food Technology, 2006. pp:99-102.

[34] Hasler C. “The changing face of functional foods”. Journal American College Nutrition, 19, 2000. pp: 499S-506S.

[35] Coronado, M., Vega, S., Gutiérrez, R., García, B., Díaz, G. “Los ácidos grasos omega-3 y omega-6: nutrición, bioquímica y salud”. REB 25(3), 2006. pp:72-79.

[36] FAO/OMS. “Grasas y aceites en la nutrición humana”. Organización Mundial de la Salud, 1997, pp: 168.

[37] Simopoulos, A. “Essential fatty acids in health and chronic disease”. Am. J. Clin. Nutr. 70, 1999. pp:560S-569S.

[38] Castor, M. “Acidos Grasos omega-3: Beneficios y Fuentes”. Interciencia, 27, 2002. pp:1-25.

[39] Denyer, G. “The renaissance of fat roles in membrane structure signal transduction and gene expression”. Med J Australia, 176, 2002. pp: S109.

[40] Calvani, M., Benatti, P. “Polyinsaturated fatty acids (PUFA)”. Sigma-Tou S.P.A. 43, 2003.

[41] Goodfellow, J., Bellamy, M., Ramse, M., Jones, C., Lewis, M. “ Dietary supplementation with marine omega-3 fatty acids improve in subjects with hypercholesterolemia”, J Am CollCardiol, 35, 2000. pp: 265-270.

[42] Contreras, M., Rapaport, S. “Recent studies on interactions between n-3 and n-6 polyunsaturated fatty acids in brain and other tissues”. CurrOpinLipidol, 13, 2002. pp:267-272.

[43] Carrero, J., Martín-Bautista, E., Baró, L., Fonollá, J., Jiménez, J., Boza, J. y López-Huertas, E. “Efectos cardiovasculares de los ácidos grasos omega-3 y alternativas para incrementar su ingesta”. Nutr. Hosp. XX, (1), 2005. pp: 63-69.

[44] Bautista, M., Castro, A., Camarena, E., Wrobel, K., Wrobel, K., Alanís, G., Sierra, Z., Da motta, V. “Desarrollo de pan Integral con Soya, Chía, Linaza y Ácido Fólico como Alimento Funcional para la Mujer”. Archivos Latinoamericanos de Nutrición, 57, (1), 2005. pp:78-84.

[45] Vioque, J.; Millán, F. “Los péptidos bioactivos en alimentación: nuevos agentes promotores de salud”. Grasas y Aceites, 52, (2), 2001. pp:5.

[46] Vioque, J., Sanchez, R., Clemente, A., Pedroche, J., Yust, M., y Millán, F. “Péptidos bioactivos en proteínas de reserva”. Grasas y Aceites, 51, 2000. pp:361-363.

[47] Fitzgerald, R. y Murray, B. “Bioactive peptides and lactic fermentations”. International Journal of Dairy Technology, 8, 2006. pp:451-457.

[48] Sun, J., He, H., y Jun, B. “Novel antioxidant peptides from fermented mushroom Ganoderma lucidum”. Journal of Agricultural and FoodChemistry, 52, 2004. pp:6646-6652.

[49] Vioque, J., Clemente, A., Pedroche, J., Yust, M., y Millán, F. “Obtención y aplicaciones de hidrolizados proteicos”. Grasas y Aceites, 52, 2001. pp:132-136.

[50] Guadix, A., Guadix, E., Páez-Dueñas, M., González-Tello, P., y Camacho, F. “Procesos tecnológicos y métodos de control en la hidrólisis de proteínas”. ArsPharmaceutica, 41, (1), 2000. pp:79-89.

[51] McCarthy, A.; O’Callagan, Y.; O’Brien, N. “Protein Hydrolysates from Agricultural Crops-Bioactivity and Potential for Functional Food Development”. Agriculture, 3, 2013. pp:112-130.

[52] Nielsen, P., Petersen, D., y Damdmann, C. “Improved method for determining food protein degree of hydrolysis”. Journal of Food Science, 66, 2001. pp:642-646.

[53] Costa, L.; Summa, M. “Soy protein in the management of hyperlipidemia”. Ann Pharmacother; 34, 2000. pp:931-935.

[54] Adams, M.; Golden, D.; Franke, A.; Potter, S.; Smith, H.; Anthony, M. “Dietary soy beta-conglycinin (7S globulin) inhibits atherosclerosis in mice”. J Nutr 134(3), 2004. pp:511–6.

[55] Desroches, S.; Mauger, J.; Ausman, L.; Lichtenstein, A.; Lamarche, B. “Soy protein favorably affects LDL size independently of isoflavones in hypercholesterolemic men and women”. J Nutr 134(3), 2004. pp:574–579.

[56] Martínez, O.; Martínez, A. “Proteínas y péptidos en nutrición enteral”. NutriciónHospitalaria, 21(2), 2006. pp: 1-14.

[57] Nagaoka, S.; Miwa, K.; Eto, M.; Kuzuya, Y.; Hori, G.; Yamamoto, K. “Soy protein peptic hydrolysate with bound phospholipids decreases micellar solubility and cholesterol absorption in rats and Caco-2 cells”. J Nutr 129, 1999. pp:1725–30.

[58] Yoshikawa, M.; Fujita, H.; Matoba, N.; Takenaka, Y.; Yamamoto, T.; Yamachi, R.; Tsuruki, H.; Takahata, K. “Bioactive peptides derived from food proteins preventing lifestyle related diseases”. BioFactors 12(1-4), 2000. pp:143–6.

[59] Revilla, E.; Maria, C.; Miramontes, E.; Bautista, J.; García, A.; Cremades, O.; Cert, R.; Parrado, J. “Nutraceutical composition, antioxidant activity and hypocholesterolemic effect of a water-soluble enzymatic extract from rice bran”. Food Res. Int. 42, 2009. pp:387-393.
[60] Ascencio, C.; Torres, N.; Isoard, F.; Gomez, F.; Hernandez, R.; Tovar, A. “Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats”. J Nutr 134(3), 2004. pp:522-529.

[61] Aoyama, T.; Fukui, K.; Takamatsu, K.; Hashimoto, Y.; Yamamoto, T. “Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (yellow KK)”. Nutrition 16(5), 2000a, pp: 349-54.

[62] Aoyama, T.; Fukui, K.; Nakamori, T.; Hashimoto, Y.; Yamamoto, T.; Takamatsu, K.; Sugano, M. “Effect of soy and milk whey protein isolates and their hydrolysate on weight reduction in genetically obese mice”. BiosciBiotechnolBiochem 64, 2000b, pp: 2594–600.