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REVIEW ARTICLE

Lipoproteins were not created by nature to cause atherosclerosis. Endogenous lipoprotein metabolism. A contemporaneous and comprehensive review for the clinician.

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ABSTRACT

Aim and scope: This review is intended to update the knowledge, but above all, to understand the difference between "normal" level vs. biological level of LDL cholesterol (LDL-C) and the physiology of the metabolism of lipoproteins with apoprotein B100 (LP-apoB100). Such medical knowledge is fundamental before introducing to the world of dyslipidemia, atherosclerosis, and pharmacotherapeutics.

Unfortunately, the teaching of the enunciated concepts -as a starting point for basic knowledge- is frequently mixed with multiple related topics, among them: the pathophysiology analysis of LP-apoB100 metabolism, that is, of dyslipidemias; the description of transgenic animal models for their study; Mendelian randomization studies of the correlation between certain genetic patterns or single nucleotide polymorphism with specific dyslipidemias and atherosclerotic cardiovascular disease (ASCVD), the study at different levels of atherosclerosis -main consequence of the abnormal metabolism of LP-apoB100- and finally, the treatment of atherogenic dyslipidemias, atherosclerosis, and ASCVD.

Hence, teaching these concepts is complex, and therefore, also the learning by the non-specialist physician of these priority chapters in modern medicine. This review is premised on the following sentence: "LP-apoB100 were not created by nature to cause atherosclerosis".

In this contemporary review we will analyze current knowledge on: the physiological value of LP-apoB100 with an emphasis on LDL, metabolism of LP-apoB100 (assembly and secretion of VLDL by the hepatocyte, circulatory transformation of VLDL to IDL, circulatory/hepatic transformation of IDL to LDL and hepatobiliary elimination of LDL) and finally LDL oxidation and elimination by reverse transport.

As Goldstein and Brown anticipated in the 1970s: "only by understanding the metabolism of LP-apoB100 will we be able to develop drugs to treat hypercholesterolemia and reduce its implicit atherosclerotic cardiovascular risk."

Introduction

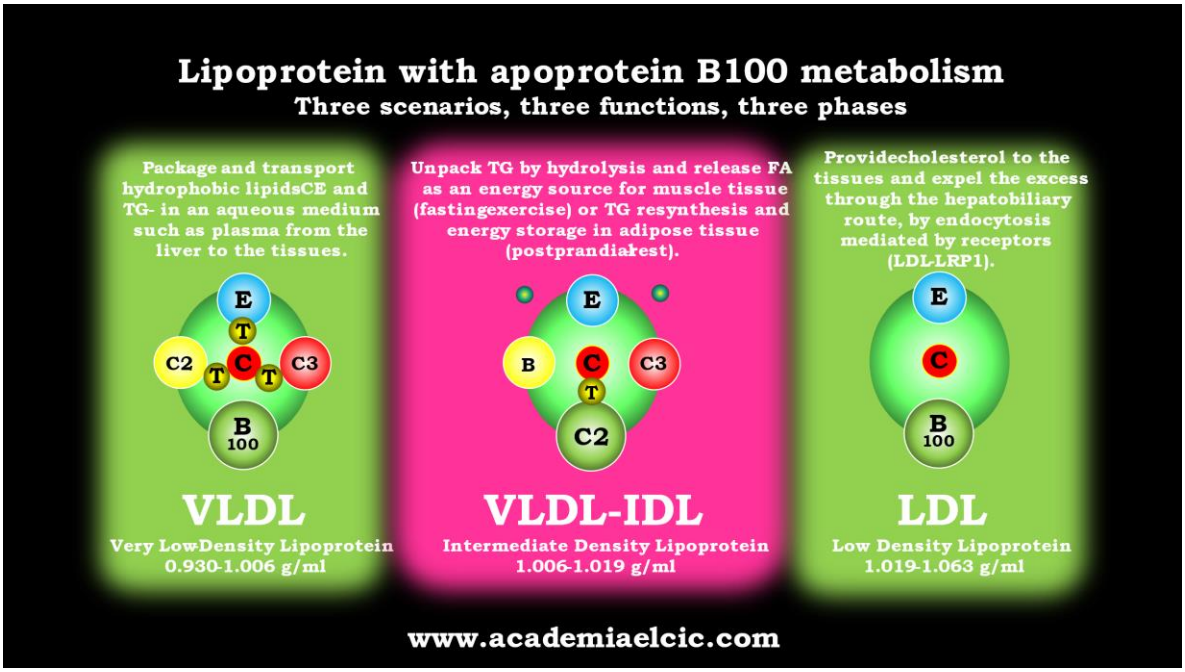
Teleologically (physiological end or purpose), LP-apoB100 are macromolecules whose biological objective is to package in the liver within very-low-density lipoproteins (VLDL) (density 0.96-1.006 g/L), essential hydrophobic lipids, especially triglycerides (TG) and esterified cholesterol. Once assembled, the hepatocyte secretes VLDL into the systemic circulation; this way, essential hydrophobic lipids (insoluble in water) can be transported from the liver to peripheral tissues in an aqueous medium such as plasma.

In the systemic circulation and influenced by the energy balance, VLDL (by hydrolysis of its triglycerides), fulfill its primary physiological purpose, releasing glycerol and especially fatty acids (FA) towards the cardiac and skeletal muscle tissues. Which, in turn, will be used as an energy substrate for beta-oxidation and adenosine triphosphate (ATP) generation or towards the adipose tissue to be used as a substrate for the resynthesis of triglycerides (adipocyte lipogenesis) and its eventual rehydrolysis with the release of fatty acids (adipocyte lipolysis).

VLDL, with lower triglyceride content, constitute intermediate-density lipoproteins or IDL, also known as VLDL remnants (density 1.007-1.019 g/L). In the systemic circulation, IDLs release glycerol and FA by hydrolysis of their remaining triglycerides, or in their hepatic circulation are rehydrolyzed by hepatic lipase (HL) or are endocytosed by hepatic LDL receptors (LDL-R) and/or LDL receptor related protein-1 (LRP1).

Rehydrolyzed and not endocytosed IDLs at the liver, with zero or minimal triglyceride content and high esterified cholesterol content, constitute low-density lipoproteins or LDL (density 1020-1060 g/L). LDLs in the systemic circulation can be endocytosed by the LDL-R of peripheral tissues that require cholesterol or, in the hepatic circulation are endocytosed by the LDL-R. A smaller percentage of LDL-C is used for cellular metabolism in the hepatocyte, and a larger percentage is eliminated through hepatobiliary reverse transport.

Thus, in the metabolism of LP-apoB00 physiology, in its first stage, different types of VLDL are assembled in the hepatocyte. In its second stage, the triglycerides contained in the VLDL are hydrolyzed in the systemic circulation, transforming these sequentially into IDL and LDL. In its final metabolic phase, LDL will deliver esterified cholesterol to the peripheral tissues cells that require it and/or will facilitate the hepatobiliary elimination of the lipid, thus fulfilling the second and third physiological purpose of LP-apoB100, namely: to provide cholesterol to the cells of the economy and eliminate excess cholesterol via the hepatobiliary pathway



Central Figure. The three stages (liver, circulation, and liver) and the three functions (assembly, transformation, and clearance) of apoB100 lipoprotein metabolism are summarized (details in text).

Physiological value of Low-Density Lipoprotein

In the 1980s, Goldstein and Brown asked themselves, **"are the LDL cholesterol levels that we consider normal today really normal, or in reality, are they excessively high?"**[1] This questioning was based on several investigations carried out in the 1970s. In 1975, based on various epidemiological studies[2-4], Keys[5] published that LDL-C levels in industrialized societies compared to non-industrialized societies were excessively high. Also establishing that LDL-C levels in non-human mammals are less than 50 mg/dL, like those of newborn human mammals, and that in the latter, the LDL-C level doubles in adolescence and quadruples in adulthood.

In 1978, the Reich and Myant group, in collaboration with the Brown and Goldstein group[6], demonstrated that LDL-R saturates with an average LDL level in plasma of 25 mg/dL, equivalent to 2.5 mg/dL in lymph, in "in vitro" studies using LDL labeled with radioactive iodine. Likewise, they demonstrated that with this level of LDL, the enzymatic activity of hydroxyl methyl glutaryl coenzyme A reductase (HMGCoAR) (a pivotal enzyme of cholesterol cellular synthesis) is completely inhibited.

This implies that the intracellular cholesterol level is the most critical variable maintained by the LDL-R homeostatic mechanism elucidated by Brown and Goldstein.[1] In other words, the decrease in the plasmatic level of LDL-C does not decrease the intracellular cholesterol level since the LDL-R has a high affinity for its ligand apoprotein B100 (apoB100). Even with a plasma LDL level of 10 mg/dL, LDL-R in peripheral tissues is 50% saturated with LDL, and endocytosis uptake of LDL-C continues unabated.

Finally, in 1979 Bilheimer[7], at that time a collaborator of Goldstein and Brown and tutor

of Grundy and Stone (collaborators of the work above and ultimately main authors of ATP III and IV), and in 1981 Kovanen,[8] confirmed that, in dogs, chimpanzees and humans, LDL production is similar (around 15 mg/kg of weight) in lipoproteins pharmacokinetic studies. However, the elimination or catabolism differs significantly between the three mammalian species.

Bilheimer and Kovanen[7-8] reported that the elimination of LDL expressed as the fractional catabolic rate (FCR), which is equivalent to the ratio between the circulating LDL pool and the eliminated LDL pool, is 1.6 in dogs, 0.8 in chimpanzees, and 0.4 in humans. This variance in FCR explains the difference between circulating levels of LDL-C (25 mg/dL in dogs, 50 mg/dL in chimpanzees, and ≥100 mg/dL in human adults.)

The mechanisms that explain the reduction in FCR in human adults, determined by the reduction in the synthesis, expression, and/or function of LDL-R, are unknown. Perhaps the most viable epigenetic hypothesis is the one originally proposed by Goldstein and Brown,[1] which alludes to high LDL-C concentrations since human intrauterine life.

More recently, genetic models have been discovered. For example, the heterozygous or homozygous loss-of-function mutation of the gene that encodes PCSK9, in which the individuals who inherited it live from the intrauterine age with LDL-C levels of less than 25 mg/dL without adverse effects.[9-10] Likewise, in individuals with hypobetalipoproteinemia due to heterozygous or homozygous loss of function of the gene that encodes apoB100, plasma LDL-C levels can be 15 mg/dL also from intrauterine life, with normal growth and development, in fact, with greater longevity.[11]

Therefore, almost four decades ago, three concepts were demonstrated to understand the difference between the physiological and "normal" values of LDL-C, atherogenesis, ASCVD, and their treatments. These concepts are the following:

a) Contemporary human adults live with an LDL-C level three to four times higher than newborn humans and non-human mammals.
b) Cholesterol is a life-essential lipid; however, all mammalian cells can synthesize it from acetate, and if required, an LDL-C level of no more than 25 mg/dL provides the supplemental cholesterol sufficient for cellular metabolism.
c) Supraphysiological levels of LDL-C (>50 mg/dL) in human adults are mainly explained by a reduction in LDL catabolism, secondary to the downregulation of synthesis, expression, and/or function of LDL-R.

Thus, the gap ≥100 mg/dL between the "normal" or average level of LDL-C (125 mg/dL) and the physiological level of LDL-C (between 25-50 mg/dL) explains much of the risk for atherosclerosis and ASCVD.

From a mechanistic point of view, Goldstein and Brown, and Steinberg and Witztum, demonstrated in the 1970s-80s that supraphysiological levels of LDL-C favor its oxidation and, with it, its transformation into molecular patterns of recognition by macrophages for its elimination via reverse transport. Hence, above a specific limit, these patterns are activators of innate and acquired immunity and initiators of the process known as atherogenesis[12].

Normal metabolism of lipoproteins with apoprotein B100.

ASSEMBLY OF VLDL IN THE HEPATOCYTE: The formation of VLDL begins in the hepatocyte with the constitutive transcription, translation, and transport towards the endoplasmic reticulum (ER) of the apoB100 encoded by the APOB gene located in the short arm of chromosome 2 (2p24.1).[13] This macro polypeptide, an essential structure of LP-apoB100 and a substrate for recognition by LDL receptors, consists of 4,536 amino acids, and its cotranslational lipidation with hydrophobic lipids, especially esterified cholesterol, and triglycerides begin in the ER and concludes in the Golgi apparatus (GA)[14-17].

In the hepatocyte, free or non-esterified cholesterol is esterified by the action of Acyl-CoA Cholesterol Acyltransferase-2 (ACAT2), encoded by the ACAT2 gene located on the long arm of chromosome 6 (6q25.3).[18] Once esterified, cholesterol integrates with apoB100, while triglycerides formed from glycerol and fatty acids from various sources are integrated with apoB100 by the action of the microsomal triglyceride transfer protein (MTTP) encoded by the MTTP gene located on the long arm of chromosome 4 (4q23)[19].

Thus, a molecule of pre-VLDL poor in triglycerides is initially assembled in the ER. Sequentially, also in the ER, pre-VLDL is transformed into a VLDL2 molecule with an average of 10,000 triglyceride molecules, a relatively high proportion of esterified cholesterol, and a diameter between 30-40 nm. Finally, between the ER and the GA, VLDL2 can be converted into a VLDL1 molecule with an average of 45,000 triglyceride molecules, a relatively minor proportion of esterified cholesterol, and a diameter between 40-70 nm. It should be noted that VLDL2 synthesis is mainly influenced by the hepatic cholesterol pool. In contrast, VLDL1 synthesis is modulated by the hepatic triglyceride pool and is suppressed (unlike VLDL2 synthesis) by the action of insulin and incretin hormones, especially glucagon-like peptide 1" (GLP1)[14-17].

In the so-called VLDL secretory pathway, in addition to the participation of ACAT2 and MTTP, within the ER/GA membrane system, multiple molecules participate (at least a dozen) with diverse actions. For example, apoB100 anchors in membrane lipidation of the ER/GA system, transfer of lipids from the hepatocyte cytoplasm to the forming VLDL, transport of these between the membranes of the ER/GA system, secretion of VLDL from the liver into the circulation and eventually recapture by the hepatocyte itself[14-17]. Among these molecules, the so-called small leucine-rich protein 1 (SLMR1) stands out, which has an essential role in the transport of VLDL in formation between the membranes of the ER/GA system.

Besides apoB100, other apoproteins synthesized and integrated into the amphipathic surface of the VLDL in formation in the hepatocyte are apoproteins E, C1, C2, C3 (apoE, apoC1, apoC2, apoC3).

APOE:

ApoE is encoded by the APOE gene on chromosome 19 (cluster 19q13.32)[20]. Its primary function serves as a substrate for recognizing LP-apoB100 by the family of LRP1 receptors and LDL-R for cellular endocytosis of lipoproteins. Apo E is also recognized by the proteoglycans of the endothelial

glycocalyx, which act as an "anchor" for the hydrolytic action of lipoprotein lipase (LPL) on the apoC2 of LP-apoB100.

APOC1:

ApoC1 is encoded by the APOC1 gene on chromosome 19 (cluster 19q13.32)[21]. Its function is related to high-density lipoproteins (HDL) and VLDL metabolism. Its actions are similar to those of apoC3 (see below), and it also inhibits the action of the cholesteryl ester transfer protein (CETP)[22].

APOC2:

ApoC2 is encoded by the APOC2 gene on chromosome 19 (cluster 19q13.32)[23]. Its essential function is to serve as a substrate for the recognition of LP-apoB100 by LPL for the hydrolysis of the triglycerides contained in them.

APOC3:

ApoC3 is encoded by the APOC3 gene on chromosome 11 (11q23.3)[24]. Its main functions are to promote hepatic assembly and secretion of VLDL; inhibit the hydrolysis of LP-apoB100 by interfering with the proteoglycans of the endothelial glycocalyx for the recognition of lipoproteins in apoE, thus limiting the hydrolytic action of LPL on apoC2; inhibit the endocytosis of LP-apoB100 interfering with the recognition, especially of the IDL in apo E by the hepatic LRP1/LDL-R.

Once assembled, VLDL2 and VLDL1 with their unique apoB100 molecule, their hydrophobic core of triglycerides (60%), esterified cholesterol (10-15%), and their hydrophobic/hydrophilic (amphipathic) outer shell composed of phospholipids, free cholesterol or not esterified and various apoproteins (apoE, apoC1, apoC2, apoC3 among the most important), are secreted by the hepatocyte into the systemic circulation via the hepatic sinusoids. The centripetal arrangement of apoC2 and the centrifugal arrangement of apoC3, as well as the lack of luminal exposure of LRP/LDL receptors in the liver sinusoids, prevent VLDL hydrolysis within the liver and promote its secretion into the systemic circulation[14-17].

CIRCULATORY TRANSFORMATION OF VLDL TO IDL/LDL:

The physiological purpose of VLDL secreted by the hepatocytes into the systemic circulation is to transport triglycerides and esterified cholesterol from the liver to the tissues, both hydrophobic (insoluble in plasma). In the lumen of the blood vessels, triglycerides contained in the VLDL are hydrolyzed with the release of one molecule of monoacylglycerol and two of FA for each molecule of triglyceride or triacylglycerol[14-17].

Physiologically, in states of fasting, physical activity, or by the action of counterregulatory hormones (ex., catecholamines, and glucagon), FA are the preferred energy substrates for muscle tissue for beta-oxidation and ATP generation. On the contrary, in postprandial, resting, or by the action of insulin, FA are not required by oxidative tissues and are taken up by adipose tissue as a substrate for triglyceride resynthesis (adipocyte lipogenesis), and eventually for their release into the circulation (adipocyte lipolysis).

The already hydrolyzed VLDL, with a more balanced content of triglycerides and cholesterol, is now called intermediate-density lipoprotein (IDL). In their hepatic circulation, these are rehydrolyzed or

"kidnapped" by LRP/LDL receptors ("liver fish net phenomenon").

The rehydrolyzed IDL, already devoid of triglycerides and mainly endowed with esterified cholesterol (60-80%), is now called low-density lipoprotein (LDL). These transport their load of esterified cholesterol to peripheral tissues. The esterified LDL cholesterol not used by the tissues will be excreted mainly through the hepatobiliary route. The delivery of esterified cholesterol to peripheral tissue cells requiring this lipid and to the hepatocyte is mediated by LDL-R-dependent LDL endocytosis.

Understanding that the processes described are dynamic and co-occur in their various stages, the most important molecules and processes that determine the circulatory transformation of VLDL to IDL and LDL are described below.

LIPOPROTEIN LIPASE:

The hydrolysis of triglycerides contained in VLDL is mainly mediated by the enzyme lipoprotein lipase (LPL), whose action is modulated by factors that we will call agonists (pro-hydrolysis) and antagonists (anti-hydrolysis). LPL is part of the family of pancreatic and hepatic lipases; it is encoded by the LPL gene located on chromosome 8 (8p21.3)[25], mainly expressed in skeletal, cardiac, and adipose muscle tissues (it is also expressed in the mammary tissue). LPL is a 443 amino acid glycoprotein with an anchoring site to the glycocalyx of the luminal endothelial membrane, a recognition site for apoC2, and a catalytic site[26].

Before secretion by myocytes and/or adipocytes, LPL matures in its ER by the action of a molecule called lipase maturation factor 1 (LMF1). Mature LPL is accompanied by the ER towards the luminal domain of the endothelial cells of the underlying tissue (muscular or adipose), as a chaperone, by the molecule called glycosyl-phosphatidyl-inositol HDL-binding protein 1 (GPIHBP1). In the glycocalyx of the luminal membrane of endothelial cells, LPL is recognized and "anchored" by heparan-sulfate and thus, having apoC2 as a cofactor is ready for its hydrolytic enzymatic action on the triglycerides contained in the core of VLDL/IDL. This action can be exerted as an LPL monomer or as LPL/GPIHBP1 dimer; this dimer also has a "protective" action versus the action of angiopoietin-like 4[26].

LPL AGONIST MODULATING FACTORS:

In addition to GPIHBPI, LPL has as an indirect agonist (it antagonizes ANGPL3) the apoprotein A5 (apoA5), also synthesized by the hepatocyte and encoded by the APOA5 gene located on chromosome 11 (cluster 11q-23.3)[27]. As we have already mentioned, LPL in monomeric or dimeric form, once anchored in the heparan sulfate matrix of endothelial cells, recognizes VLDL through its recognition domain for apoC2. After that, LPL initiates the hydrolysis of triglycerides through its catalytic domain. Each triglyceride molecule is transformed into a monoacylglycerol molecule, and two FA molecules are released into the circulation, where they bind to albumin or are captured by the membrane transporters cassette-domain 36 (CD36) of the overlying endothelial cells and transferred to the interstitium that nourishes the cells that require them, especially myocytes or adipocytes[26].

LPL ANTAGONISTIC MODULATING FACTORS:

The flow of monoacylglycerol, especially FA, into skeletal or myocardial muscle tissues or adipose tissue is not random. The systemic energy state influences it and depends on molecules known as angiopoietin-like.

As mentioned, fasting, physical activity, and counterregulatory hormones (catecholamines and glucagon), a state of low energy input and/or high energy demand, favor the flow of FA towards the skeletal muscle and myocardial tissues for oxidation and ATP generation. On the contrary, the postprandial state, rest, and insulin, equivalent to a state of high energy input and/or low energy demand, inhibit the flow of FA toward the oxidative tissues, thus limiting the risk of lipotoxicity and favoring the flow of FA towards the adipose tissue for the resynthesis of triglycerides[14-17].

The molecules involved in this physiological differential flow of FA are the angiopoietins "like" 3, 4, and 8 or ANGPL3/4/8. These molecules involved in the metabolism of LP-apoB100 are part of a family of eight angiopoietin-like, named for their structural similarity to the angiogenic proteins known as angiopoietins[28]. Due to its current importance as a therapeutic target[29], the characteristics and functions of angiopoietins 3, 4, and 8 are detailed below.

ANGPL3:

ANGPL3 was discovered in 1999 by Conklin et al.[28] This angiopoietin is synthesized almost exclusively by the hepatocyte; it is encoded by the ANGPL3 gene located on chromosome 1 (1p31.3)[30]. ANGPL3 consists of 3 domains: the signaling domain or peptide, the N-terminal or catalytic domain, and the C-terminal or fibrinogen-like domain.

Once secreted by the hepatocyte, ANGPL3 circulates and, in endocrine form, targets preferentially LPL of cardiac and active skeletal myocytes tissues. The function of ANGPL3 is to inhibit the action of LPL and thereby reduce VLDL/IDL hydrolysis. The loss of function of this angiopoietin determines an increase in LPL activity, greater VLDL/IDL hydrolysis, and a lower circulating concentration of triglycerides. Conversely, ANGPL3 gain-of-function causes reduced LPL activity, less VLDL/IDL hydrolysis, and higher circulating triglyceride concentrations.[28-29].

Of great importance is that, in addition to inhibiting LPL, ANGPL3 also inhibits the hydrolytic action of endothelial lipase (EL), whose primary substrate is the HDL and IDL phospholipids. Due to this action, the loss-of-function of this angiopoietin determines an increase in EL activity, greater hydrolysis of HDL and IDL phospholipids, and a lower circulating concentration of HDL-C, IDL-C, and its derivative LDL-C. Conversely, ANGPL3 gain-of-function results in reduced EL activity decreased hydrolysis of HDL and IDL phospholipids, and increased circulating concentrations of HDL-C, IDL-C, and LDL-C. It has also been suggested that, through non-enzymatic mechanisms, LPL facilitates LDL endocytosis by hepatic LDL-R. This action contributes, together with the lower transformation of IDL to LDL determined by EL, to the reduction of LDL-C[31-32].

ANGPL3 recognizes and inhibits the hydrolytic action of LPL through its N-terminal domain, which heparin, GPBIHBPI, and triglyceride-rich lipoprotein (TRL) inhibit. The intrinsic mechanism of ANGPL3 inhibition on LPL has yet to be fully understood. However, the formation of the ANGPL3-8 complex seems essential for a

strong inhibition of LPL by ANGPL3. The intrinsic mechanism of EL inhibition by ANGPL3 has yet to be studied[28-29,31-32].

ANGPL4:

ANGPL4 was discovered simultaneously in the year 2000 by Yoon and Kersten and colleagues[28]. This angiopoietin is mainly synthesized by the adipocyte; the ANGPL4 gene encodes it on chromosome 19 (19p13.2)[33]. ANGPL4, like ANGPL3, consists of 3 domains: the signaling domain or peptide, the N-terminal or catalytic domain, and the C-terminal or fibrinogen-like domain.

Once secreted by the adipocyte, ANGPL4 in autocrine and paracrine form has LPL of the adipocyte tissue as a preferential target. The function of ANGPL4 is to strongly inhibit the action of LPL and thereby reduce LRT hydrolysis. The loss-of-function of this angiopoietin determines an increase in LPL activity, greater hydrolysis of TRL, and a lower circulating concentration of triglycerides. Conversely, ANGPL4 gain-of-function causes reduced LPL activity, less TRL hydrolysis, and higher circulating triglyceride concentrations[28].

ANGPL4 is also expressed in other tissues, for example, brown adipose tissue (it is involved in the regulation of thermogenesis), cardiac and skeletal myocytes, intestine, liver, and macrophages. The inhibiting effects of ANGPL4 on LPL in such tissues are under extensive investigation. Of particular interest is the inhibitory effect of ANGPL4 on the LPL of macrophages; this inhibition downregulates lipid uptake by said cells. ANGPL4 inhibition in macrophages causes an increase in the function of macrophage LPL with the formation of Touton cells by fusion of foamy macrophages, which are highly proinflammatory[28].

ANGPL4, like ANGPL3, recognizes and inhibits the hydrolytic action of LPL through its N-terminal domain, which, in turn, is also inhibited by heparin, GPBIHBPI, and TRL. The intrinsic mechanism of LPL inhibition by ANGPL4 has also yet to be fully understood[28].

ANGPL8:

ANGPL8 was discovered simultaneously in 2012 by Quagliarini, Zhang, Kim, and colleagues[28]. This angiopoietin is mainly synthesized by hepatocytes and adipocytes; it is encoded by the ANGPL8 gene, also located on chromosome 19 (19p13.2)[34]. ANGPL8, like ANGPL3-4, consists of 3 domains: the signaling domain or peptide, the N-terminal or catalytic domain, and the C-terminal or fibrinogen-like domain. Unlike ANGPL3-4, ANGPL8 does not act by itself but through its secretion as interactive pairs ANGPL3+8 and ANGPL4+8.

Once secreted by the hepatocyte, the endocrine binomial ANGPL3+8 (ratio 3:1) has the LPL of cardiac and active skeletal myocytes (in contractile activity) as its preferential target. The function of the ANGPL3+8 binomial is to inhibit the action of LPL with greater or synergistic efficiency and thereby strongly reduce the hydrolysis of TRL. The loss-of-function of this pairing of angiopoietins determines a significant increase in LPL activity, greater TRL hydrolysis, and lower circulating concentration of triglycerides. On the contrary, the gain-of-function of the binomial causes a profound reduction in LPL activity, less hydrolysis of TRL, and a higher circulating concentration of triglycerides[28].

On the other hand, once secreted by the adipocyte, the ANGPL4+8 binomial (ratio 1:1) in autocrine and paracrine form has, as a preferential target, the LPL of the adipocytes

themselves. The function of the ANGPL4+8 binomial is to inhibit with lower or reduced efficiency (ANGPL8 acts as an ANGPL4 partial antagonist) the action of LPL and thereby moderately reduce the hydrolysis of TRL. The loss-of-function of this pairing of angiopoietins determines an attenuated increase in LPL activity, moderate TRL hydrolysis, and a lower circulating concentration of triglycerides. Conversely, the gain-of-function of the binomial results in an attenuated reduction in LPL activity, moderate TRL hydrolysis, and increased circulating triglyceride concentrations[28].

ENERGETIC STATE AND ACTION OF ANGPL3, 4, AND 8:

The dynamics of angiopoietins depend on their tissue location and energetic state. According to the current model proposed by Zhang in 2016 or the ANGPL3-4-8 model[35], fasting induces the synthesis of ANGPL4 by the adipocyte. With this, the action of LPL is inhibited at this level directing the flow of TRL into oxidative tissues. On the contrary, ingestion induces the synthesis of ANGPL3 by the hepatocyte. With this, the action of LPL is inhibited, especially at the myocardial and skeletal muscle level, reducing the risk of muscle lipotoxicity due to FA and directing the flow of TRL towards the storage tissues. This model of great physiological importance has been confirmed and extended with the following concepts:

a.- In the first hours of fasting (negative energy balance), the adipocyte synthesizes and secretes ANGPL4, which, in an autocrine and paracrine manner, intensely inhibits adipocyte LPL, facilitating the flow of TRL-TG-FA towards cardiac muscle and/or active skeleton muscle tissues. Simultaneously, in fasting, the hepatocyte inhibits the synthesis of ANGPL8 and activates the synthesis of ANGPL3; the latter, in the absence of the ANGPL3+8 binomial in an endocrine manner, inhibits myocyte LPL in an attenuated manner.[28] **The net balance of both processes favors the flow of TRL and its hydrolysis to and in oxidative tissues to form ATP.**

b.- In the first hours of ingestion (positive energy balance), the hepatocyte synthesizes the ANGPL3+8 binomial, which, in an endocrine form, intensely inhibits myocyte LPL, inhibiting the hydrolysis of TRL in said territory and facilitating the flow of TRL-TG - FA towards adipose tissue. Simultaneously, on ingestion, the adipocyte inhibits the synthesis of ANGPL4 and activates the synthesis of ANGPL8, facilitating the hydrolytic action of adipocyte LPL[28]. **The net balance of both processes favors the flow of TRL and its hydrolysis to and in storage tissues for triglyceride resynthesis. In parallel, the risk of FA lipotoxicity in oxidative tissues is attenuated.**

This way, after its hepatic secretion, in its systemic circulation, the VLDL, through the participation of multiple agonist and antagonist molecules, fulfills its first physiological objective, transport triglycerides and release monoacylglycerol and FA in an "intelligent" way towards the consuming tissues or fatty acids storage according to the systemic energy status.

INTRAHEPATIC CIRCULATORY TRANSFORMATION OF IDL AND FORMATION OF LDL:

After its primary systemic circulation, on its return to the hepatic circulation, IDL enters through the hepatic sinusoids with wide

fenestrations and is rich in heparan sulfate and LRP and LDL-R receptors. The basolateral membrane domain of hepatic sinusoidal endothelial cells is in intimate contact with the basolateral membrane domain of hepatocytes via the space of Disse or perisinusoidal interstitial space.

In a process similar to the one described for LPL, IDLs can be rehydrolyzed by hepatic lipase (HL or LIPC), a hydrolase encoded by the LIPC gene located on chromosome 15 (15q21. 3)[36]. releasing monoacylglycerol and fatty acids into the space of Disse, that are captured and integrated by the hepatocyte for VLDL resynthesis. Through the hepatic sinusoids, IDL can also be recognized as apoE by the LRP and LDL receptors of the hepatocyte and be endocytosed for its intrahepatocyte catabolism, in which the apoprotein components, especially apoE, are recycled by the hepatocyte for reuse.

Some authors have called this intrahepatic process a "liver fish net phenomenon" phenomenon. The rehydrolyzed IDLs that "survive" the described phenomenon lack triglycerides, lose apoC2/3, and are rich in esterified cholesterol. These LP-apoB100 are known as LDL due to their low density and are the protagonists of the following section.

HEPATOBILIARY ELIMINATION OF LDL: Considering that in the body, only 7% of cholesterol is circulating (70% contained in LDL), and 93% is found in the membranes and inside the cells, Goldstein and Brown formulated this fundamental question in their research on Familial Hypercholesterolemia (FH) in 1972: **¿how to reduce the level of circulating cholesterol, mainly the LDL content, without affecting the intracellular cholesterol content?** Answering this question took them four years of research and ultimately, thirteen years later, earned them the Nobel Prize in Physiology and Medicine[37.-39.].

Goldstein and Brown published the discovery of LDL-R in 1974, clarifying how our body eliminates 70% of circulating LDL-C through the liver without compromising the intracellular cholesterol content. The other 30% of LDL-C is eliminated by various scavenger receptors of cells of the reticuloendothelial system (SR-A, CD-36, SR-B1, CD-68, SR-PSOX, LOX, etc). In addition to discovering LDL-R and its life cycle, Goldstein and Brown confirmed that mutations of one or both alleles of the LDL-R-encoding gene, located on chromosome 19, were the cause of severe hypercholesterolemia in individuals with the FH phenotype and anticipated that its manipulation could influence the incidence of ASCVD even in individuals with "normal" LDL-C levels[12, 37-39].

Goldstein and Brown postulated that the increase in LDL-R synthesis, expression, and/or function might be "the master key" to answering their original question. This postulate found resonance with the discovery of compactin by Akira Endo et al. in 1976, a potent inhibitor of cellular cholesterol synthesis[40-44].

LDL RECEPTOR:

The intracellular cholesterol concentration (especially in the membranes of the ER/GA system) determines the synthesis of LDL-R and, indirectly, the concentration of circulating LDL-C. The lower the concentration of intracellular cholesterol, the higher the synthesis of LDL-R and the lower the concentration of circulating LDL-C. This

"cholesterol-mediated feedback" concept was the guideline for Goldstein and Brown to discover LDL-R and, consequently, its mutations that cause FH. These discoveries earned them the Nobel Prize in Physiology and Medicine in 1985[38].

LDL-R mobilizes 70% of circulating LDL-C and is encoded by the LDL-R gene on chromosome 19 (19p13.2)[45]. This receptor is a glycoprotein with 839 amino acids and five domains, namely[37]. a) Binding domain to its ligand; this domain recognizes apoB100 as a ligand and apoE. b) Domain like the epidermal growth factor (EGF), this domain directs the LDL-R towards the cell membrane; it is now known to be the ligand recognized by PCSK9. c) Domain rich in oligosaccharides; this domain acts as an "extender" of the LDL-R. d) Transmembrane domain; this domain "stabilizes" the LDL-R in the cell membrane. e) Intracytoplasmic domain; this domain acts as an anchor for LDL-R in membrane vesicles covered by the clathrin protein. The LDL/LDL-R binomial endocytosis initiates in these structures, known as clathrin holes or nests.

REGULATION OF LDL-R EXPRESSION:

As mentioned, the reduction in free or non-esterified cholesterol concentration in the ER/GA membranes is the trigger to activate the synthesis of HMGCoA-R encoded by the HMGCoA-R gene located in chromosome 5 (5p14.3)[46] and, therefore, cholesterol synthesis. Likewise, it activates the synthesis of LDL-R. The activation mechanism for synthesizing HMGCoA-R and LDL-R is regulated by the transcription factor Sterol Regulator Element Binding Protein 2 (SREBP-2).

Under physiological conditions, with an appropriate concentration of free cholesterol in the ER/GA membranes, SREBP-2 remains bound in the ER to its "anchor" protein called SREBP Cleavage Activating Protein (SCAP). Reducing free cholesterol in the ER/GA membranes activates Serine Proteases 1/2 (S1P/S2P). In the first step, S1P proteolyzes the SCAP/SREBP-2 binding, thus releasing SREBP-2 from its binding to the SCAP protein. In the second step, S2P proteolyzes the SREBP-2/ER binding, thus releasing SREBP-2 from its ER binding[47]. This way, SREBP-2 can migrate from the ER to the GA and to the nucleus, activating the genes that code for HMGCoA-R and LDL-R synthesis.

LDL-R FUNCTION:

Once encoded and synthesized, LDL-R migrates to the basolateral membrane of the hepatocyte in contact with the space of Disse. At this location, its recognition domain for apoB100 captures circulating LP-apoB100, especially LDL, and initiates the process of endocytosis[37-39, 48-50].

From the clathrin-rich membrane vesicles, the LDL/LDL-R binomial is transferred to cytoplasmic structures known as endosomes, which, by fusing their membranes with the lysosome membranes, transfer their LDL/LDL-R content to them. Inside the endolysosome, the pH becomes acidic, and the dissociation between LDL-R and LDL is facilitated due to the action of various ATPases. LDL remains inside the lysosome for catabolism, and LDL-R migrates to the cell membrane to repeat the cycle.

On average, an LDL-R has a half-life of 20 hours, a membrane-endosome-membrane cycle takes approximately 10 minutes, and each LDL molecule carries 1,500-2,000 esterified cholesterol molecules. This means that each LDL-R captures from the circulation

between 180,000 and 240,000 molecules of esterified cholesterol contained in LDL. Undoubtedly a receptor with very high efficiency. Three intracellular protein complexes have recently been described (CCC, WASH, and retriever) that also regulate LDL-R recycling[51].

INTRACELLULAR CATABOLISM OF LDL:

LDL contained in the endosomes is hydrolyzed. The protein component releases amino acids into the cytoplasm, which will be used for cell metabolism, and the lipid component (especially esterified cholesterol, once hydrolyzed) is released into the cytoplasm as free or non-esterified cholesterol and can have various metabolic destinations:

a) It can be directed towards the ER/GA membranes to compensate for the process that initiates the synthesis of LDL-R, that is, the depletion of free cholesterol in the membranes of the ER/GA system. This transport is carried out by a process known as "hands-off," recently described by Brown and Goldstein[37].

b) The action of ACAT-2 can esterify it, and thus, it is stored in the cytoplasm as "drops" of esterified cholesterol.

c) It can be eliminated "in reverse" towards the bile canaliculi of the hepatobiliary circulation by the membrane transporters, ATP Binding Cassette G5 and G8 (ABCG5/8), located in the apical membrane of the hepatocyte. A variable amount of cholesterol eliminated into the hepatobiliary circulation can be reabsorbed into the hepatocyte cytoplasm by the Nieman-Pick protein C1L1 (NPC1L1) of the hepatocyte bile membrane.

LRP RECEPTORS:

Within the family of receptors for lipoproteins, there are many receptors, the so-called LDL Receptor Related Protein type 1 (LRP1) encoded by the LRP gene located on chromosome 12 (12q13.3)[52]. This receptor has a structure similar to LDL-R and recognizes multiple ligands, especially recognizes apoE (abundant apoprotein in chylomicron remnant lipoproteins apoB48) as a ligand; it also recognizes apoE and apoB100 of VLDL and IDL. Its principal function is to act in the liver as a purifying receptor for chylomicron remnants and VLDL or IDL.

PCSK9:

Pro-protein Convertase Subtilisin Kexin type 9 or PCSK9, originally called Neuronal Apoptosis Regulator Convertase type 1 (NARC-1), was described in 2013 by Nabil Seidah et al[53-54].

This protease is the ninth member of the family of serine-proteases or subtilases. These proteins are serine-proteases that regulate the activation, inactivation, and/or intracellular translation of secretory proteins such as transcription or growth factors, prohormones, membrane receptors, etc. There are nine identified subtilases, seven from the Kexin subfamily in bacteria and fungi and two from the Kexin-like subfamily in mammals. In humans, SK1/S1P subtilase regulates the activity of the transcription factors SREBP and BDNF, and PCSK9 subtilase regulates LDL-R activity.

The PCSK9 gene encodes PCSK9 on chromosome 1 (1p32.3)[55]. The PCSK9 primordium or pre-pro-PCSK9 is synthesized in the liver, intestine, and kidney as an inactive 692 amino acid glycoprotein with four domains. Amino acids 1-30 make up the signal peptide or domain; amino acids 31-152, the propeptide or inhibition domain;

amino acids 153-452, the Subtilisin-like peptide or catalytic domain and amino acids 453-692, the Cysteine-rich peptide or C-terminal domain[56].

Thanks to two autocatalytic steps, prepro-PCSK9 is transformed into PCSK9 in the ER. In the first step, it loses the signaling domain and the inhibition domain in the second. The resulting molecule, or PCSK9 with 540 amino acids and two domains, the catalytic or Subtilisin-like, and the C terminal, is secreted and circulates free in the plasma; its average concentration ranges from 50-600 ng/mL[56].

THE BIOLOGICAL FUNCTION OF PCSK9:
PCSK9, like LP-apoB100, are not molecules designed to cause atherosclerosis. PCSK9 is a biological regulator of its substrate, LDL-R, and is teleologically designed to prevent intracellular cholesterol overload, a feasible situation if cellular cholesterol synthesis and its uptake by LDL-R function without counter-regulation. Thus, we can say that LDL-R and PCSK9 are Yin-Yang in the intracellular regulation of cholesterol[56].

Incorporating esterified cholesterol into the cell via LDL-R is finely regulated by PCSK9. As we have already reviewed, the concentration of free cholesterol in ER/GA is the biological variable that regulates the expression of the transcription factor SREBP-2. SREBP-2 is activated after a reduction in free cholesterol in ER/GA. According to what has already been reviewed, the activation of this transcription factor determines the following biological processes:

1. Activates the synthesis of HMGCoAR for the de novo synthesis of cholesterol.
2. Activates the synthesis of LDL-R for the uptake of circulating LDL cholesterol.
3. In extreme cases activates cellular autophagy

These processes, whose objective is to keep the intracellular free cholesterol content constant, have a counter-regulation mechanism that prevents the cell from being "flooded" with cholesterol. This counter-regulation mechanism activates the synthesis of PCSK9, which is triggered " in parallel" by the same transcription factor SREBP-2 acting on the PCSK9 gene.

PCSK9 is secreted into the circulation once synthesized and "prepared" for secretion by losing its signaling and inhibition domains. In circulation, it recognizes domain 2 or the EFG-like domain of LDL-R as a ligand. Once this recognition is done, in the presence and even in the absence of LDL, the PCSK9/LDL-R/LDL trinomial or the PCSK9/LDL-R binomial is endocytosed by the process seen above. The binding of PCSK9 to LDL-R prevents domain 2 of the latter from directing the migration or recycling of LDL-R towards the membrane, which causes the PCSK9/LDL-R binomial to be hydrolyzed within the endosomes, thus balancing the intake and intracellular cholesterol content[57-66].

LDL oxidation and elimination by reverse transport.

In 1979, Goldstein and Brown demonstrated that in vitro peritoneal macrophages rapidly took up that native LDL modified by acetylation. However, LDL acetylation in vivo has never been demonstrated[12].

In the 1980s, various research groups, especially the work team of Steinberg and Witztum in La Jolla, California[12], demonstrated that the oxidation of LDL during its exposure

to endothelial cells in culture-generated oxidized LDL (ox-LDL) was avidly taken up by macrophages. Based on this finding, multiple research groups have shown that ox-LDL are molecules with homology to the wall of Gram-positive bacteria and to the membrane of cells in apoptosis, which, like ox-LDL, activate in macrophages the expression of scavenger receptors such as SR-A, CD-36, SR-B1, CD-68, SR-PSOX, and LOX-1. The phagocytosis of ox-LDL by the macrophage allows an average uptake of 25% of the circulating LDL and eliminates them via reverse cholesterol transport[12].

Ox-LDL is generated from native LDL by the action of various oxidative enzyme systems, including lipoxygenases, myeloperoxidases, NADPH-oxidase, and uncoupled nitric oxide synthase (NO-synthase)[12].

Beyond the facilitating biological role of reverse cholesterol transport, macrophages that phagocytose ox-LDL, with the participation of the major histocompatibility system, present oxidized LDL to other cell lines, especially lymphocytes that produce antibodies of the IgM, IgG type, and lymphokines. This way, especially the oxidized phosphocholine of ox-LDL is recognized as a damage-associated molecular pattern (DAMP) or pathogens (PAMP) with the capacity to activate the innate and acquired immune response[12].

The inability, due to macrophage supersaturation, to mobilize excess ox-LDL by reverse transport will lead to the formation of macrophages overloaded with ox-LDL, also called foamy macrophages, a cell line characteristic of the initial phases of the physiopathology and pathology of atherogenesis, a topic not addressed in this review and extensively reviewed by the author[67-68].

Conclusion

Thus, starting from our original premise that lipoproteins were not created by nature to cause atherosclerosis, we have reviewed the difference between the "normal" value and the physiological or biological value of LDL-C (25-50 mg/dL). Physiologically, this circulating level of LDL-C is maintained by the delicate balance between VLDL production, its transformation to IDL and LDL, and especially by LDL-R-mediated hepatic uptake and hepatobiliary elimination of cholesterol contained in LDL.

These processes, known as endogenous metabolism of LP-apoB100, meet three physiological objectives, namely: a) to provide efficient energy substrates (FA) to cells with high energy consumption (myocytes) and energy storage cells (adipocytes); b) to provide cholesterol (metabolic and structural substrate) to cells whose synthesis is not sufficient to cover their metabolic and/or structural requirements, for this purpose, 25 mg/dL of circulating LDL-C is sufficient, and c) to capture at the liver the LDL that is not used by peripheral tissues and eliminate its cholesterol content by reverse hepatobiliary transport, the hepatocyte is the only cell in the body with this physiological capacity. A smaller percentage of circulating LDL-C is modified by oxidation, taken up by tissue macrophages, and eliminated by reverse transport through HDL (now reviewed).

The imbalance in any of these physiological processes, that is to say, the increase in the hepatic production of VLDL, the reduction of its circulatory transformation towards IDL and

LDL, and especially, the reduction in the elimination of the latter "waste from the metabolism of LP- apoB100" through the hepatobiliary route, are the pathophysiological substrate that underlies atherogenic dyslipidemias, atherogenesis and finally ASCVD, the leading causes of disability, death, and health expenditure in contemporary humans who have lived since childhood with supraphysiological levels of LDL-C

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