REVIEW ARTICLE

Lipotoxicity, the role in the process of atherosclerosis

Jiayao Li[a], Xiao Zhang[b], Zixuan Xing[c], Yixin Sun[d], Shengyan Cui[e], Xintao Lv[f], Shuaiwei Guo[g], Liqun Jiao[h], Wenjing Li[i], Tao Wang[j]

Article history:
Received: 01-08-2024
Revised: 28-08-2024
Accepted: 15-09-2024
Published: 01-10-2024

[a] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.
This author contributed equally to this work.

[b] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.
This author contributed equally to this work.

[c] Health Science Center, Xi'an Jiaotong University, Shaanxi, China.

[d] First Hospital, Peking University, Beijing, China.
Health Science Center, Peking University, Beijing, China.

[e] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.

[f] Capital Medical University, Beijing, China, 100053.

[g] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.

[h] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.
Department of Interventional Radiology, Xuanwu Hospital, Capital Medical University, Beijing, China
Corresponding Author:
liqunjiao@sina.cn

[i] Laboratory of Computational Biology and Machine Intelligence, School of Artificial Intelligence, University of Chinese Academy of Sciences, Beijing, 100049, China.
State Key Laboratory of Multimodal Artificial Intelligence Systems, Institute of Automation, Chinese Academy of Sciences, Beijing, 100190, China
Corresponding Author:
wenjing.li@ia.ac.cn

[j] Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China.
China International Neuroscience Institute (China-INI), 45 Changchun Street, Beijing, 100053, China.
Corresponding Author:
wangtao_dr@sina.com

ABSTRACT

Background: Atherosclerosis (AS) is a growing problem in the elderly population causing a variety of diseases with high mortality and disability rates. Lipotoxicity plays an important role in the process of AS.

Aim: This review aims to summarize the characteristics of glucose and lipid metabolism, local pathological changes of arteries and functional changes or death of perivascular cells in the process of lipotoxicity.

Result: From the perspective of lipotoxicity and aging, we review the current understanding of the relationship between lipid metabolism and the development of atherosclerosis, and discuss the corresponding pharmacological treatment options.

Conclusion: Various metabolic factors can lead to lipotoxicity, which in turn affects the vascular wall and various cells within the blood vessels, leading to the development of atherosclerosis. Clarifying the role of lipotoxicity in atherosclerosis provides a new perspective for the prevention and treatment of atherosclerosis.

Keywords: Lipotoxicity; Atherosclerosis; Aging; Inflammation.

INTRODUCTION

Lipids play vital roles in various physiological processes. High-fat causes metabolic disorders, cell dysfunction and cell death, which is called lipotoxicity (1). Under physiological conditions, as free fatty acids (FFA) accumulate more than needed in tissues and organs of the human body, the excess fat is esterified and stored in lipid droplets as triglycerides and mobilized by cellular lipases when needed (2). Compared with adipose tissue, which can store excess fatty acids in the cytoplasm, the ability of non-adipose cells to store spilled fat is relatively limited. (2). Long-term uptake of excessive fat, insulin resistance, excess circulating natural and oxidized low-density lipoprotein (LDL) levels, oxidative stress and other factors can cause excessive FFA that can not be stored and increase FFA uptaken by various organs, leading to lipotoxicity.

Atherosclerosis is a chronic inflammatory and immune disease which can cause acute coronary syndrome, ischemic stroke, transient ischemic attack, aneurysm and other vascular diseases (3,4). This review aims to summarize the current regulation and prevention of lipotoxicity, the role of lipotoxicity in the course of atherosclerosis, as well as feasible and valuable therapeutic targets (5).

1. LIPOTOXICITY & PRODUCTION OF FFA-INDUCED LIPOTOXICITY

1.1. Lipotoxicity

As mentioned above, cells of nonadipose tissue have a limited capacity to store lipids, and as the capacity is overwhelmed, cellular dysfunction, metabolic disorders, and inflammatory response will occur, sometimes leading to cell death, which is called lipotoxicity (6). Lipotoxicity also leads to abnormal changes in the levels of various metabolites, which play a role in the development of atherosclerosis. One of the bases of lipotoxicity is dyslipidemia. When lipotoxicity occurs, cholesterol level normally shows a trend of decreasing in high-density lipoprotein cholesterol (HDLc) level and increasing in low-density lipoprotein cholesterol (LDLc) level.

1.2. Production of FFA-induced lipotoxicity

1.2.1. Reactive oxygen species

Under physiological conditions, intracellular FFA regulates intracellular reactive oxygen species (ROS) production through various mechanisms (7). As FFA increases, especially the polyunsaturated fatty acids and palmitic acid content, the overall trend shows an increase in ROS production. The related mechanisms include adipose tissue paracrine cytokine Tumor necrosis factor α (TNF- α) directly activating nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase to increase ROS production in vascular smooth muscle cells (VSMCs) (8) and stimulating mitochondria to produce O_2^-. (9-12). FFA can slow down the electron flow through complexes I and III through the interaction within the complex subunit structure. The isolated mitochondrial oxidative complex I-related substrates respond to FFA by increasing the production of O_2^-., and at the same time, when electrons are transferred directly from succinate (in the presence of rotenone) to complex III, FFA strongly promotes the formation of complex III-related O_2^-. (7,13-15). The interference of FFA on the electron transport of the respiratory chain may be achieved by disrupting the balance of the respiratory supercomplex. In addition to the direct inhibition of FFA on the electron transport chain, FFA can also stimulate the production of ROS by depleting cytochrome c in mitochondria (16-19), thus blocking the electron flow from complex III to complex IV. In addition, it is worth noting that the dissipation of palmitate dependence of $\Delta \psi m$ is parallel to the increase of mitochondrial ROS production. The components of FFA strongly promote the production of ROS (Figure 1).

1.2.2. Oxidized phospholipids

When lipotoxicity increases intracellular oxygen free radicals and inflammation of perivascular cells, phospholipids form oxidized phospholipids (OxPls) through non-enzymatic and enzymatic oxidation. Via various signal pathways, OxPls affects cell inflammatory response and cell phenotypes to participate in the occurrence and development of atherosclerosis. (13-17)

In plasma, most of OxPls comes from lipoprotein (a) (LP (A)), while a small part of OxPls comes from apoptotic cell membrane and oxidized low-density lipoprotein (oxLDL) particles (18). In cells, phospholipids in the lipoproteins and membrane are readily induced by free radicals, while free radical-induced lipid peroxidation (LPO) occurs at the sn2 site (19). There are more than 30 bioactive types of oxidized phospholipids in atherosclerotic species. We only take 1-palmitoyl-2-arachidonoyl-sn-3-glycero-phosphorylcholine (PAPC) as an example to explain the occurrence of phospholipase-catalyzed and non-enzymatic oxidation. The polyunsaturated fatty acid (PUFA) at the sn2 site of this type of oxidized phospholipid is arachidonic acid (AA).

Enzymatic oxidation means that PAPC produces free AA under the catalysis of phospholipase A2, and then eicosanes are formed under the catalysis of lipoxygenase (LOX), cyclooxygenase (COX) and cytochrome P450. The compounds obtained from this process regulate inflammatory response through various signal pathways.

Non-enzymatic oxidation refers to the process in which ROS acts on phospholipids to produce oxidized phospholipids. ROS from non-enzymatic sources (smoking, air pollution and other environmental factors) and enzyme sources (such as NADPH oxidase) mainly act on phospholipids containing polyunsaturated fatty acids. (Fig. 1)

1.2.3. NO

NO plays an important role in the development of atherosclerosis (3). FFA has a negative impact on the generation and function of NO. Long-term and persistent elevated FFA damages essential NO

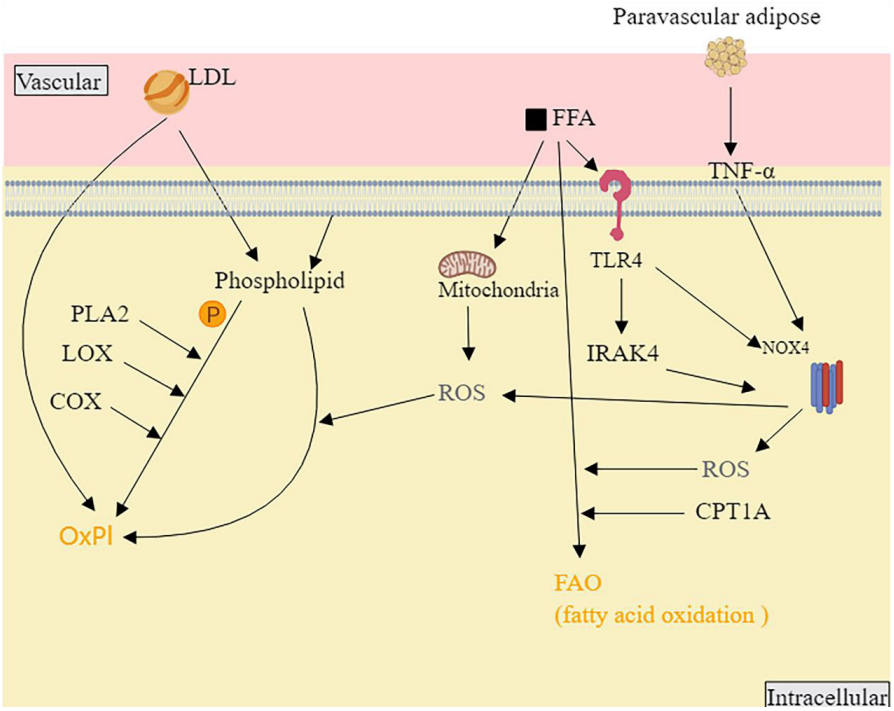

Figure 1. This figure illustrates the pattern by which lipotoxicity leads to increase in intracellular superoxide levels. After oxidation, phospholipids can be divided into full-length and truncated phosphoric acid. Through various cell receptors and signal pathways, OxPls affect the gene expression of vascular parietal cells to promote the occurrence, development or inhibition of inflammation in the pathogenesis of atherosclerosis. TLR4: Toll-like receptor 4; IRAK4: Interleukin-1 receptor-associated kinase 4; NOX4: NADPH oxidase 4; CPT1A: Carnitine palmitoyltransferase 1A; PLA2: Phospholipase A2.

production and insulin-mediated NO production in endothelial cells in a dose and time-dependent manner, which has been confirmed in both human and in vitro experiments (20). By incubating bovine aortic endothelial cells (BAECs) with saturated fatty acid palmitate / oleic acid, the transcription factor nuclear factor kappa-B (NF-κB) signal pathway is activated in human and bovine endothelial cells through serine kinase inhibitor of kappa B kinase (IKK) under palmitate treatment and subsequent insulin stimulation. This regulator of the inflammatory pathway may disrupt insulin signaling by inhibiting the phosphorylation of insulin receptor substrate-1 (IRS-1), protein kinase B (Akt) and endothelial nitric oxide synthases (eNOS), resulting in a reduction in NO production (21-23).

2. ROLE OF FATTY ACIDS IN THE PROCESS OF ATHEROSCLEROSIS

In the pathological process of lipotoxic AS, abnormally elevated FFA in circulation, especially saturated fatty acids (SFA), can lead to lipotoxicity in various types of vascular cells. This would cause phenotypic transformation, calcification, loss of nitric oxide (NO) secretion, enhanced inflammatory response, endothelium-dependent vasodilation, insulin resistance, time-dependent apoptosis and so on (24,25). At the same time, elevated lipid levels, along with increased ROS and insulin resistance, form a feedback loop that participates in the disease progression of AS.

2.1. Effect of lipotoxicity on blood vessels

When lipids and fatty acids accumulate in vascular endothelium, as mentioned above, in addition to the decrease of endothelial NO production and dysfunction directly caused by lipotoxicity, increased ROS production and insulin resistance produce the same pathophysiological effects. Due to the combined effects, NO-dependent vasodilation, local anti-inflammatory responses, inhibition of endothelial

and smooth muscle cell proliferation, as well as antioxidant and antiplatelet activities, are all reduced, thereby further accelerating the progression of atherosclerosis (26).

2.2. Lipotoxicity contributes to AS by affecting multiple cell types

2.2.1. Effects of lipotoxicity on VSMCs

The abnormal proliferation of VSMCs promotes atherosclerotic plaque formation, while in the progressive stage, it increases plaque stability. Correspondingly, increasing macrophages and foam cells in the plaque will reduce the plaque stability. However, recent genetic pedigree tracking studies have shown that VSMCs may be the source of macrophage-like and foam cells. They can also aggravate plaque calcification when VSMCs change the phenotype (27).

With oxidized phospholipid activation, the expression of Krüppel-like factor 4 (KLF4) increases. KLF4 binds to the G/C suppressor element of most smooth muscle cell (SMC) marker gene promoters such as actin alpha 2 (Acta2), transgelin (Tagln), and myosin heavy chain 11 (MYH11) (28-31), resulting in a decrease in the expression of KLF4-dependent SMC markers and an increase in the expression of macrophage-like cells. In addition, cholesterol load increases with the enhancement of KLF4-dependent phagocytosis (31). In the mouse model, inhibitors like tamoxifen are used to simulate partial inhibition of KLF4. Although it could not prevent the phenotypic transition of VSMCs, it could reduce the lesion area of AS by about 30%, increase plaque stability, reduce intra-plaque bleeding and reduce cell proliferation and apoptosis (27,31).

Palmitic acid (PA) can also activate the transformation of osteogenic genes to osteogenic phenotypes by activating Acyl-CoA synthetase long chain family member 3 (ACSL3). Real-time fluorescence polymerase chain reaction (Real-time PCR) showed that the mRNA levels of embryonic bone differentiation factor bone morphogenetic protein-2 (BMP-2), osteogenic transcription factor msh homeobox 2 (MSX2) and bone matrix protein osteopontin increased significantly after PA treatment. As further proof, through the use of acyl-CoA synthase (ACS) inhibitors or siRNA to completely block the induction of osteogenic genes BMP-2 and MSX2 by PA, adenovirus-mediated ACSL3 overexpression enhanced the expression of BMP-2 and MSX2 induced by PA, indicating that the activation of osteogenic genes by PA depends on its activation of ACSL3 (2) (Fig. 2).

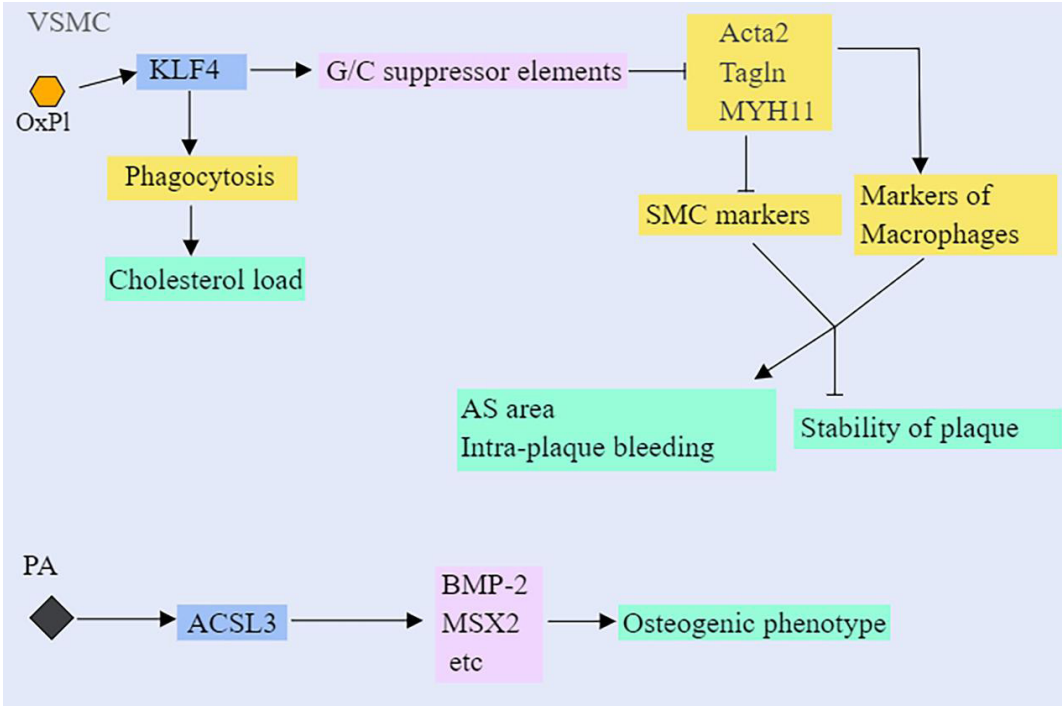

Figure 2. In VSMC, OxPl and PA affect the transformation of VSMCs.

2.2.2. Effects of lipotoxicity on macrophages

Macrophages are sensitive to lipotoxicity. As lipids are increased, macrophages influenced by lipotoxicity contribute to the pathogenesis of several metabolic derangements (32-34). When the level of palmitic acid in macrophages increases abnormally, it will cause endoplasmic reticulum stress. The expression of unfolded protein response (URP) markers can be detected in macrophages in both human and mouse atherosclerotic lesions (35,36). The endoplasmic reticulum stress induced by PA in macrophages depends at least partly on the spliced XBP-1 (sXBP-1) and C/EBP homologous protein (CHOP) (37), which are two elements involved in the transcriptional process induced by UPR. In vitro experiments, after PA-induced macrophages, endoplasmic reticulum stress was confirmed by detecting the level of protein kinase-like ER kinase (PERK) and eukaryotic initiation factor 2 (eIF-2) phosphorylation. Adapter protein 2 (AP2) protein is up-regulated rapidly and significantly during endoplasmic reticulum stress, and the immunostaining of AP2 can be substantially inhibited after co-treatment with 4-phenyl butyric acid (PBA) (37,38). AP2 participates in lipid-induced endoplasmic reticulum stress and is inhibited by PBA. Further experiments in vitro showed that PA could not induce endoplasmic reticulum stress in AP2 deficient (AP2-/-) macrophages. More specifically, it could not induce the expression of URP target genes recombinant RNA damage-inducible transcript 3 (Ddit3) and sXBP-1. Although tunicamycin could induce endoplasmic reticulum stress in this kind of macrophages, AP2 deficient macrophages still had the ability to endoplasmic reticulum stress (37). However, the regulatory effect of AP2 on lipid-induced endoplasmic reticulum stress depends on its lipid binding activity. For AP2 deficient cells with recombinant lipid binding receptors, even if AP2 is recombined with it, it still shows strong resistance to endoplasmic reticulum stress. Correspondingly, AP2-/- macrophages also significantly prevented PA-induced apoptosis, considering the inhibition of caspase-3 activity and poly ADP-ribose polymerase (PARP) cleavage.

At the same time, under the condition of free cholesterol load, although the cholesterol influx of wildtype (WT) and AP2-/- cells was not significantly different, even the cholesterol influx of AP2-/- was higher than that of WT macrophages, free cholesterol (FC)-induced endoplasmic reticulum stress only appeared in WT macrophages.

2.2.3. Effects of lipotoxicity on endothelial cells

OxPl activates cAMP/PKA, which leads to increasing expression of P-selection and finally enhances the rolling of leukocytes and initiates recruitment (39). Some types of OxPl play an essential role in the process of inflammation. 1-palmitoyl-2-glutaroylsnglycero-phosphocholine (PG-PC) will increase the level of E-selection and vasopressin-activated $Ca2^{+}$-mobilizing (VACM-1), which enhances the interaction of monocyte-endothelial and neutrophil-endothelial. On the contrary, 1-palmitoyl-2-oxovalaroyl-snglycero-phosphocholine (POV-PC) inhibits the expression of E-selection and interaction of neutrophil-endothelial and enhances monocyte-endothelial interaction, consequently leading to chronic inflammation (40). After POV-PC and 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphocholine (PEI-PC) bind cellular surface receptors, increasing the level of cyclic Adenosine Monophosphate (cAMP), cAMP induces activation of ras-related protein (R-RAS). This will directly promote the expression of α5β1integrin induced by phosphoinositide 3-kinase (PI3K), which further leads to the deposition of connecting segment 1 (CS-1) fibronectin on the cell surface (41). (Fig. 3).

FFA can cause lipotoxicity and endothelial dysfunction of endothelial cells through various mechanisms, including decreased secretion of NO stimulated by insulin, aggravation of inflammatory response in endothelial cells and injury of endothelial-dependent vasodilation.

In the mouse carotid artery ligation model, emulsified ethyl palmitate was rapidly decomposed into palmitate after intraperitoneal injection, which strongly aggravated the formation of neointima, and the neointimal lesion was mainly composed of phenotypic regulated SMCs (42).

In the experiment of Steinbergetal *et al.,* Intralipid 2h injection attenuated the vasodilation induced by acetylcholine. This effect of FFA did not depend on chain length or prostaglandin synthesis (43). The same effect was also seen in the decreased response to 5-Hydroxytryptamine (5-HT) in patients with high triglyceride (44).

PA can also cause endoplasmic reticulum stress in endothelial cells, and endoplasmic reticulum stress can lead to the transformation of endothelial cells into mesenchymal cells (45). After endothelial cells were treated with tunicamycin or thapsigargin, the endothelial cell marker platelet

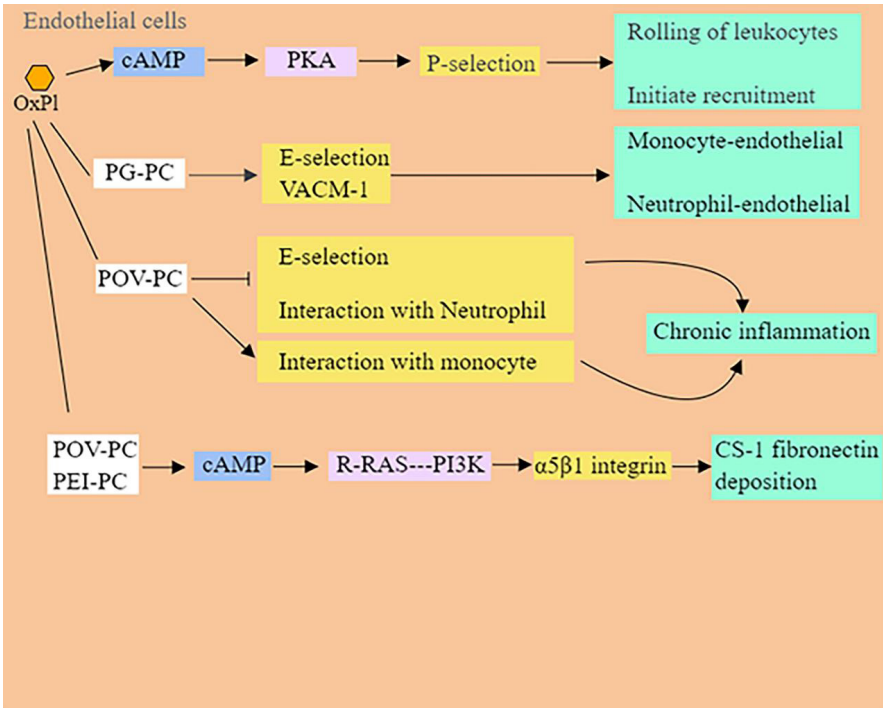

Figure 3. In endothelial cells, OxPl play an essential role in inflammation.

endothelial cell adhesion molecule-1 (CD31) was significantly deleted, while the fibroblast markers α-SMA, Vimentin and collagen 1 were increased considerably. Endothelial cell-mesenchymal cell transformation induced by endoplasmic reticulum stress depends on the Src pathway. After using Src kinase inhibitor and treated with tunicamycin in vitro, the expression of CD31 and vascular endothelial growth factor receptor 2 (VEGFR-2) was significantly up-regulated, while the expression of α-SMA and Vimentin was down-regulated. At the same time, the use of Smad blockers could not achieve the same effect (45).

3. LIPID METABOLISM

3.1 Cholesterol

One of the bases of lipotoxicity is dyslipidemia. When lipotoxicity occurs, cholesterol level normally shows a trend of a decrease in high-density lipoprotein cholesterol (HDLc) level and an increase in low-density lipoprotein cholesterol (LDLc) level. Cholesterol is an absolute need to maintain the function of human cells. However, high intracellular cholesterol levels can lead to lipotoxicity. Since most peripheral cells can not break down cholesterol, cholesterol efflux is significant for lipid balance, and efflux defects can lead to lipotoxicity and atherosclerotic plaques (46). Therefore, regulating intracellular free cholesterol is essential to reduce or avoid lipotoxicity.

There are four main mechanisms of cholesterol clearance in cells: water diffusion, scavenger receptor class B, type I (SR-BI)-mediated diffusion, ATP-binding cassette transporter A1 (ABCA1)-mediated efflux and ATP-binding cassette transporter G1 (ABCG1)-mediated efflux (47-49). It should be noted that the importance of each mechanism in cholesterol assignment is different between the physiological state and fat load state. Take macrophages for example, passive diffusion contributes 80% of the lipid efflux of macrophages at the physiological state, meaning simple diffusion is a major contributor to cellular cholesterol outflow to the serum (46,49,50). Yet, under the condition of lipid load, ABCA1-mediated efflux contributes 40-50% of cholesterol outflow, and the proportion of passive diffusion decreases to 30% (46,47,50). In both cases, different degrees of mechanism contribution provide possible therapeutic targets.

3.2. Free fatty acids

When lipolysis occurs, free fatty acids are released from adipose tissue and several cell types

(51). Saturated fatty acids with 13-21 carbons are defined as long-chain saturated fatty acids (LCSFA) (52). With the condition of obesity, increase of LCSFA uptake, overload of fat storage capacity or loss of fat tissue expansion, the level of circulating FFA will increase (53). In addition, central obesity, diabetes, and insulin resistance increase circulating FFA levels as well. With the chronic increase of circulating FFA, insulin resistance in muscle and liver will further aggravate, leading to a vicious circle (54).

As the circulating FFA level increases, the body can counteract lipid overload through a series of compensatory responses: 1. storing of triglycerides in the form of lipid droplets; 2. oxidation procedure of activating mitochondria and peroxisome; 3. remodeling of membrane lipid in the cell membrane, including sphingolipid formation and functional membrane microdomain (or "lipid raft") remodeling (55). As FFA plays one of the most crucial roles in lipotoxicity, the following part mainly takes FFA as an example to explain the role of lipotoxicity (LP) in AS.

Under physiological conditions, fatty acids enter cells via passive diffusion and transporters on the cell membrane, including palmitoylated Platelet glycoprotein 4 (CD36), fatty acid transport proteins (FATP) and fatty acid-binding protein (FABP), which are caveolae sunken structures in the cell membrane (56). CD36 can also bind modified lipids and internalized particles to promote oxidized low-density lipoprotein (ox-LDL) uptake, which plays a crucial role in macrophage cholesterol accumulation. (57,58)

In the pathological process of lipotoxicity, after CD36-mediated phagocytosis of ox-LDL, the expression of CD36 will increase (59), and ox-LDL uptake will further increase. There is no negative feedback regulation in this cycle; cell foaming will be accelerated, and peroxisome proliferators-activated receptor γ (PPAR γ) and Nuclear factor erythroid2-related factor 2 (Nrf2) will be activated to promote the activation of CD36 further, resulting in the formation of AS (60,61). Among them, the activation of PPAR γ is related to the appearance of intracellular short-chain fatty acids and their derivatives and a lipid signal network is established between the nucleus and the cell surface (1). PPAR γ can enhance CD36, while Nrf2 increases recruitment to the Sterol-regulatory element binding proteins1 (SRBEP-1) promoter after lipotoxicity-induced oxidative stress (62).

4. PROSPECTS OF PHARMACOLOGICAL DRUGS TARGETING LIPOTOXICITY

4.1. Classical treatment

Statins that have been put into clinical use can protect patients from atherosclerosis and lipotoxicity by increasing the level of circulating HDLc. At the same time, some non-statins, such as bile acid isolators, niacin and fibrates, can reduce circulating LDLc, but nicotinic acid and fibrates can not be combined with statins (63). In a kind of free triglycerides in circulation, this can be achieved by inactivating angiogenin-like 3, an inhibitor of lipoprotein lipase (LPL), by genetic or drug means (64). In addition, lipotoxicity is often accompanied by metabolic syndrome or insulin resistance, so in addition to the direct treatment related to lipid metabolism, the treatment of glucose metabolism and insulin resistance, as well as dietary guidance to patients, play an important role in treatment.

4.2. PPAR Agonists

Statins are able to interact with PPARs, which partly explains their beneficial effects. Based on this fundamental truth (65), PPAR therapies in the context of obesity and diabetes have rapidly developed. For example, Thiazolidinediones (TZDs) and fibrates (PPAR-a agonists) are potentially favorable candidates for treating diabetic nephropathy (66). On the other hand, adverse effects associated with TZD treatment, such as fluid retention, cardiovascular complications, and bone loss, among others, have limited their clinical use (67).

4.3. Adiponectin Receptor Agonists

As adiponectin decreases in the context of metabolic syndrome, it leads to fatty acid accumulation (68). Respectively, to exert the activity of adiponectin, it binds with its receptors, AdipoR1 and AdipoR2. Adiponectin is an essential target against lipotoxicity-mediated harmful effects (69). For example, AdipoRon, an orally active synthetic adiponectin agonist, can reduce lipotoxicity and improve insulin resistance and obesity-related disease (70-73).

4.4 VEGF-B Signaling inhibition Vascular endothelial growth factor B (VEGF-B), a foremost responsible for lipid control in endothelial cells (74,75), via its union with receptors located on the cell surface as Neuropilin-1 and VEGFR1 induces

the expression of the fatty acid transport proteins FATP3 and FATP4, favoring lipid accumulation (76). Thus, modulation of VEGF-B signaling like VEGF-B prevents dyslipidemia and insulin resistance, reducing lipid accumulation (77).

5. CONCLUSION

High-fat diet, metabolic syndrome, insulin resistance and other factors can lead to lipotoxicity. Lipotoxicity can affect the functional and phenotypic transformation of vascular smooth muscle cells, endothelial cells and macrophages by affecting the production of various intracellular products and gene activation. Clarifying lipotoxicity's mechanism and signal pathway on vascular cells provides a new starting point for treating and preventing atherosclerosis.

Acknowledgements

The authors would like to express their gratitude to colleges in Xuanwu hospital, for providing the necessary guidance and support to finish. This research would not have been possible without their cooperation and participation.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China [82301468], and the Incubating Program of Capital Medical University [PYZ22041] awarded to TW, the National Natural Science Foundation of China [82171303] and Beijing Hospitals Authority's Ascent Plan [DFL20220702] awarded to LJ. ♦

REFERENCES

1. Thakur M, Tupe RS. Lipoxin and glycation in SREBP signaling: Insight into diabetic cardiomyopathy and associated lipotoxicity. Prostaglandins Other Lipid Mediat. 2023 Feb;164:106698.
2. Kageyama A, Matsui H, Ohta M, Sambuichi K, Kawano H, Notsu T, *et al*. Palmitic acid induces osteoblastic differentiation in vascular smooth muscle cells through ACSL3 and NF-κB, novel targets of eicosapentaenoic acid. PloS One. 2013;8(6):e68197.
3. Bir SC, Kelley RE. Carotid atherosclerotic disease: A systematic review of pathogenesis and management. Brain Circ. 2022 Sep 21;8(3):127-36.
4. Al Kasab S, Hess DC, Chimowitz MI. Rationale for ischemic conditioning to prevent stroke in patients with intracranial arterial stenosis. Brain Circ. 2016;2(2):67-71.
5. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, *et al*. Atherosclerosis. Nat Rev Dis Primer. 2019 Aug 16;5(1):56.
6. Lim S, Meigs JB. Ectopic fat and cardiometabolic and vascular risk. Int J Cardiol. 2013 Nov 5;169(3):166-76.
7. Schönfeld P, Wojtczak L. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radic Biol Med. 2008 Aug 1;45(3):231-41.
8. De Keulenaer GW, Alexander RW, Ushio-Fukai M, Ishizaka N, Griendling KK. Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. Biochem J. 1998 Feb 1;329 (Pt 3)(Pt 3):653-7.
9. Srivastava S, Chan C. Hydrogen peroxide and hydroxyl radicals mediate palmitate-induced cytotoxicity to hepatoma cells: relation to mitochondrial permeability transition. Free Radic Res. 2007 Jan;41(1):38-49.
10. Dymkowska D, Szczepanowska J, Wieckowski MR, Wojtczak L. Short-term and long-term effects of fatty acids in rat hepatoma AS-30D cells: the way to apoptosis. Biochim Biophys Acta. 2006 Feb;1763(2):152-63.
11. Fauconnier J, Andersson DC, Zhang SJ, Lanner JT, Wibom R, Katz A, *et al*. Effects of palmitate on Ca(2+) handling in adult control and ob/ob cardiomyocytes: impact of mitochondrial reactive oxygen species. Diabetes. 2007 Apr;56(4):1136-42.
12. Rachek LI, Musiyenko SI, LeDoux SP, Wilson GL. Palmitate induced mitochondrial deoxyribonucleic acid damage and apoptosis in l6 rat skeletal muscle cells. Endocrinology. 2007 Jan;148(1):293-9.
13. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition

of a common ligand: Phosphorylcholine of oxidized phospholipids. Proc Natl Acad Sci U S A. 2002 Oct 1;99(20):13043-8.
14. Chang MK, Bergmark C, Laurila A, Hörkkö S, Han KH, Friedman P, *et al*. Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. Proc Natl Acad Sci U S A. 1999 May 25;96(11):6353-8.
15. Kagan VE, Gleiss B, Tyurina YY, Tyurin VA, Elenström-Magnusson C, Liu SX, *et al*. A role for oxidative stress in apoptosis: oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis. J Immunol Baltim Md 1950. 2002 Jul 1;169(1):487-99.
16. Berliner JA, Subbanagounder G, Leitinger N, Watson AD, Vora D. Evidence for a role of phospholipid oxidation products in atherogenesis. Trends Cardiovasc Med. 2001;11(3-4):142-7.
17. Berliner J. Introduction. Lipid oxidation products and atherosclerosis. Vascul Pharmacol. 2002 Apr;38(4):187-91.
18. Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: insights from epidemiology, genetics, and biology. J Lipid Res. 2016 Nov;57(11):1953-75.
19. Hörkkö S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G, *et al*. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. J Clin Invest. 1999 Jan;103(1):117-28.
20. Karman RJ, Gupta MP, Garcia JG, Hart CM. Exogenous fatty acids modulate the functional and cytotoxic responses of cultured pulmonary artery endothelial cells to oxidant stress. J Lab Clin Med. 1997 May;129(5):548-56.
21. Kim F, Tysseling KA, Rice J, Pham M, Haji L, Gallis BM, *et al*. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. Arterioscler Thromb Vasc Biol. 2005 May;25(5):989-94.
22. Maloney E, Sweet IR, Hockenbery DM, Pham M, Rizzo NO, Tateya S, *et al*. Activation of NF-kappaB by palmitate in endothelial cells: a key role for NADPH oxidase-derived superoxide in response to TLR4 activation. Arterioscler Thromb Vasc Biol. 2009 Sep;29(9):1370-5.
23. Symons JD, McMillin SL, Riehle C, Tanner J, Palionyte M, Hillas E, *et al*. Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure. Circ Res. 2009 May 8;104(9):1085-94.
24. Artwohl M, Lindenmair A, Roden M, Waldhäusl WK, Freudenthaler A, Klosner G, *et al*. Fatty acids induce apoptosis in human smooth muscle cells depending on chain length, saturation, and duration of exposure. Atherosclerosis. 2009 Feb 1;202(2):351-62.
25. Mesenchymal stem cells alleviate palmitic acid-induced endothelial-to-mesenchymal transition by suppressing endoplasmic reticulum stress | American Journal of Physiology-Endocrinology and Metabolism [Internet]. [cited 2023 Aug 3]. Available from: https://journals.physiology.org/doi/full/10.1152/ajpendo.00155.2020?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org
26. Imrie H, Abbas A, Kearney M. Insulin resistance, lipotoxicity and endothelial dysfunction. Biochim Biophys Acta. 2010 Mar;1801(3):320-6.
27. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res. 2016 Feb 19;118(4):692-702.
28. Regan CP, Adam PJ, Madsen CS, Owens GK. Molecular mechanisms of decreased smooth muscle differentiation marker expression after vascular injury. J Clin Invest. 2000 Nov;106(9):1139-47.
29. Salmon M, Gomez D, Greene E, Shankman L, Owens GK. Cooperative binding of KLF4, pELK-1, and HDAC2 to a G/C repressor element in the SM22α promoter mediates transcriptional silencing during SMC phenotypic switching in vivo. Circ Res. 2012 Aug 31;111(6):685-96.
30. Wamhoff BR, Hoofnagle MH, Burns A, Sinha S, McDonald OG, Owens GK. A G/C element mediates repression of the SM22alpha promoter within phenotypically modulated smooth muscle cells in experimental atherosclerosis. Circ Res. 2004 Nov 12;95(10):981-8.
31. Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, *et al*. KLF4 Dependent Phenotypic Modulation of SMCs Plays a Key Role in Atherosclerotic Plaque Pathogenesis. Nat Med. 2015 Jun;21(6):628-37.
32. Aqel NM, Ball RY, Waldmann H, Mitchinson MJ. Monocytic origin of foam cells in human

atherosclerotic plaques. Atherosclerosis. 1984 Dec;53(3):265-71.
33. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol. 2008 Dec;8(12):923-34.
34. Gregor MF, Hotamisligil GS. Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. J Lipid Res. 2007 Sep;48(9):1905-14.
35. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol. 2005 Nov;25(11):2255-64.
36. Myoishi M, Hao H, Minamino T, Watanabe K, Nishihira K, Hatakeyama K, *et al*. Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. Circulation. 2007 Sep 11;116(11):1226-33.
37. Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow M, *et al*. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med. 2009 Dec;15(12):1383-91.
38. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, *et al*. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006 Aug 25;313(5790):1137-40.
39. Vora DK, Fang ZT, Liva SM, Tyner TR, Parhami F, Watson AD, *et al*. Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. Circ Res. 1997 Jun;80(6):810-8.
40. Leitinger N, Tyner TR, Oslund L, Rizza C, Subbanagounder G, Lee H, *et al*. Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. Proc Natl Acad Sci U S A. 1999 Oct 12;96(21):12010-5.
41. Cole AL, Subbanagounder G, Mukhopadhyay S, Berliner JA, Vora DK. Oxidized phospholipid-induced endothelial cell/monocyte interaction is mediated by a cAMP-dependent R-Ras/PI3-kinase pathway. Arterioscler Thromb Vasc Biol. 2003 Aug 1;23(8):1384-90.
42. Eguchi K, Manabe I. Toll-Like Receptor, Lipotoxicity and Chronic inflammation: The Pathological Link Between Obesity and Cardiometabolic Disease. J Atheroscler Thromb. 2014;21(7):629-39.
43. de Kreutzenberg SV, Crepaldi C, Marchetto S, Calò L, Tiengo A, Del Prato S, *et al*. Plasma free fatty acids and endothelium-dependent vasodilation: effect of chain-length and cyclooxygenase inhibition. J Clin Endocrinol Metab. 2000 Feb;85(2):793-8.
44. de Man FH, Weverling-Rijnsburger AW, van der Laarse A, Smelt AH, Jukema JW, Blauw GJ. Not acute but chronic hypertriglyceridemia is associated with impaired endothelium-dependent vasodilation: reversal after lipid-lowering therapy by atorvastatin. Arterioscler Thromb Vasc Biol. 2000 Mar;20(3):744-50.
45. Ying R, Wang XQ, Yang Y, Gu ZJ, Mai JT, Qiu Q, *et al*. Hydrogen sulfide suppresses endoplasmic reticulum stress-induced endothelial-to-mesenchymal transition through Src pathway. Life Sci. 2016 Jan 1;144:208-17.
46. Castaño D, Rattanasopa C, Monteiro-Cardoso VF, Corlianò M, Liu Y, Zhong S, *et al*. Lipid efflux mechanisms, relation to disease and potential therapeutic aspects. Adv Drug Deliv Rev. 2020;159:54-93.
47. Phillips MC. Molecular Mechanisms of Cellular Cholesterol Efflux *. J Biol Chem. 2014 Aug 1;289(35):24020-9.
48. Favari E, Chroni A, Tietge UJF, Zanotti I, Escolà-Gil JC, Bernini F. Cholesterol Efflux and Reverse Cholesterol Transport. In: von Eckardstein A, Kardassis D, editors. High Density Lipoproteins: From Biological Understanding to Clinical Exploitation [Internet]. Cham: Springer International Publishing; 2015 [cited 2023 Sep 25]. p. 181-206. (Handbook of Experimental Pharmacology). Available from: https://doi.org/10.1007/978-3-319-09665-0_4
49. Anastasius M, Kockx M, Jessup W, Sullivan D, Rye KA, Kritharides L. Cholesterol efflux capacity: An introduction for clinicians. Am Heart J. 2016 Oct 1;180:54-63.
50. Adorni MP, Zimetti F, Billheimer JT, Wang N, Rader DJ, Phillips MC, *et al*. The roles of different pathways in the release of cholesterol from macrophages. J Lipid Res. 2007 Nov 1;48(11):2453-62.
51. Rodríguez-Carrio J, Salazar N, Margolles A, González S, Gueimonde M, de Los Reyes-Gavilán CG, *et al*. Free Fatty Acids Profiles Are Related to Gut Microbiota Signatures and Short-Chain Fatty Acids. Front Immunol. 2017;8:823.
52. Hidalgo MA, Carretta MD, Burgos RA. Long Chain Fatty Acids as Modulators of Immune

Cells Function: Contribution of FFA1 and FFA4 Receptors. Front Physiol. 2021;12:668330.
53. Lemos G de O, Torrinhas RS, Waitzberg DL. Nutrients, Physical Activity, and Mitochondrial Dysfunction in the Setting of Metabolic Syndrome. Nutrients. 2023 Feb 28;15(5):1217.
54. Jensen MD. Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model. Obes Silver Spring Md. 2006 Feb;14 Suppl 1:20S-24S.
55. Carobbio S, Rodriguez-Cuenca S, Vidal-Puig A. Origins of metabolic complications in obesity: ectopic fat accumulation. The importance of the qualitative aspect of lipotoxicity. Curr Opin Clin Nutr Metab Care. 2011 Nov;14(6):520-6.
56. Hao JW, Wang J, Guo H, Zhao YY, Sun HH, Li YF, *et al*. CD36 facilitates fatty acid uptake by dynamic palmitoylation-regulated endocytosis. Nat Commun. 2020 Sep 21;11(1):4765.
57. Febbraio M, Podrez EA, Smith JD, Hajjar DP, Hazen SL, Hoff HF, *et al*. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. J Clin Invest. 2000 Apr;105(8):1049-56.
58. Zeng Y, Tao N, Chung KN, Heuser JE, Lublin DM. Endocytosis of oxidized low density lipoprotein through scavenger receptor CD36 utilizes a lipid raft pathway that does not require caveolin-1. J Biol Chem. 2003 Nov 14;278(46):45931-6.
59. Yazgan B, Sozen E, Karademir B, Ustunsoy S, Ince U, Zarkovic N, *et al*. CD36 expression in peripheral blood mononuclear cells reflects the onset of atherosclerosis. BioFactors Oxf Engl. 2018 Nov;44(6):588-96.
60. Chistiakov DA, Bobryshev YV, Orekhov AN. Macrophage-mediated cholesterol handling in atherosclerosis. J Cell Mol Med. 2016 Jan;20(1):17-28.
61. Feng L, Gu C, Li Y, Huang J. High Glucose Promotes CD36 Expression by Upregulating Peroxisome Proliferator-Activated Receptor γ Levels to Exacerbate Lipid Deposition in Renal Tubular Cells. BioMed Res Int. 2017;2017:1414070.
62. Sun X, Li X, Jia H, Wang H, Shui G, Qin Y, *et al*. Nuclear Factor E2-Related Factor 2 Mediates Oxidative Stress-Induced Lipid Accumulation in Adipocytes by Increasing Adipogenesis and Decreasing Lipolysis. Antioxid Redox Signal. 2020 Jan 20;32(3):173-92.
63. Writing Committee, Lloyd-Jones DM, Morris PB, Ballantyne CM, Birtcher KK, Daly DD, *et al*. 2016 ACC Expert Consensus Decision Pathway on the Role of Non-Statin Therapies for LDL-Cholesterol Lowering in the Management of Atherosclerotic Cardiovascular Disease Risk: A Report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol. 2016 Jul 5;68(1):92-125.
64. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, *et al*. Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. N Engl J Med. 2017 Jul 20;377(3):211-21.
65. Sharma RB, Alonso LC. Lipotoxicity in the pancreatic beta cell: not just survival and function, but proliferation as well? Curr Diab Rep. 2014 Jun;14(6):492.
66. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation. 1998 Nov 10;98(19):2088-93.
67. Jia Z, Sun Y, Yang G, Zhang A, Huang S, Heiney KM, *et al*. New Insights into the PPAR γ Agonists for the Treatment of Diabetic Nephropathy. PPAR Res. 2014;2014:818530.
68. Choi HM, Doss HM, Kim KS. Multifaceted Physiological Roles of Adiponectin in Inflammation and Diseases. Int J Mol Sci. 2020 Feb 12;21(4):1219.
69. Saxena NK, Anania FA. Adipocytokines and hepatic fibrosis. Trends Endocrinol Metab TEM. 2015 Mar;26(3):153-61.
70. Zha D, Wu X, Gao P. Adiponectin and Its Receptors in Diabetic Kidney Disease: Molecular Mechanisms and Clinical Potential. Endocrinology. 2017 Jul 1;158(7):2022-34.
71. Yamakado S, Cho H, Inada M, Morikawa M, Jiang YH, Saito K, *et al*. Urinary adiponectin as a new diagnostic index for chronic kidney disease due to diabetic nephropathy. BMJ Open Diabetes Res Care. 2019;7(1):e000661.
72. Lin J, Hu FB, Curhan G. Serum adiponectin and renal dysfunction in men with type 2 diabetes. Diabetes Care. 2007 Feb;30(2):239-44.
73. Saraheimo M, Forsblom C, Fagerudd J, Teppo AM, Pettersson-Fernholm K, Frystyk J, *et al*. Serum adiponectin is increased in type 1 diabetic patients with nephropathy. Diabetes Care. 2005 Jun;28(6):1410-4.
74. Muoio DM. Metabolism and vascular fatty acid transport. N Engl J Med. 2010 Jul 15;363(3):291-3.

75. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, *et al*. Vascular endothelial growth factor B controls endothelial fatty acid uptake. Nature. 2010 Apr 8;464(7290):917-21.
76. Hagberg C, Mehlem A, Falkevall A, Muhl L, Eriksson U. Endothelial fatty acid transport: role of vascular endothelial growth factor B. Physiol Bethesda Md. 2013 Mar;28(2):125-34.
77. Hagberg CE, Mehlem A, Falkevall A, Muhl L, Fam BC, Ortsäter H, *et al*. Targeting VEGF-B as a novel treatment for insulin resistance and type 2 diabetes. Nature. 2012 Oct 18;490(7420):426-30.