

REVIEW

This paper is dedicated to the 70th anniversary of the founding of Physiologia Bohemoslovaca (currently Physiological Research)

Adipose Tissue Macrophages and Atherogenesis – A Synergy With Cholesterolaemia

Rudolf POLEDNE¹, Ivana KRALOVA LESNA^{1,2}

¹Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, ²Anaesthesiology, Resuscitation and Intensive Care Unit, Military University Hospital, Prague, Czech Republic

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Summary

Excessive LDL cholesterol concentration together with subclinical inflammation, in which macrophages play a central role, are linked pathologies. The process starts with the accumulation of macrophages in white adipose tissue and the switch of their polarization toward a pro-inflammatory phenotype. The proportion of pro-inflammatory macrophages in adipose tissue is related to the main risk predictors of cardiovascular disease. The cholesterol content of phospholipids of cell membranes seems to possess a crucial role in the regulation of membrane signal transduction and macrophage polarization. Also, different fatty acids of membrane phospholipids influence phenotypes of adipose tissue macrophages with saturated fatty acids stimulating pro-inflammatory whereas ω 3 fatty acids anti-inflammatory changes. The inflammatory status of white adipose tissue, therefore, reflects not only adipose tissue volume but also adipose tissue macrophages feature. The beneficial dietary change leading to an atherogenic lipoprotein decrease may therefore synergically reduce adipose tissue driven inflammation.

Key words

Inflammation • Adipose tissue macrophages • Plasma membrane • Cholesterol • ω 3 fatty acids

Corresponding author

Rudolf Poledne, IKEM, Videnska 1958/9, 140 21 Prague, Czech Republic. E-mail: rudolf.poledne@ikem.cz

Introduction

Atherosclerosis develops as the result of a pathological interplay between increased inflow of

cholesterol (either from LDL particles or lipoprotein remnants) into the arterial wall and local recruitment of monocytes (Tabas and Lichtman 2017). While cholesterol transport from the liver to extrahepatic tissue is critical for cell membrane formation, common plasma cholesterol levels in industrialized countries exceed physiological needs. This physiological mechanism worked satisfactorily for the overwhelming majority of individuals until the late 19th century when LDL-cholesterol concentration increased due to substantial increase of animal fat consumption. The effect of this negative dietary change was nicely documented by long-term analysis of the lifespan in a large Dutch family with familial hypercholesterolemia (Sijbrands *et al.* 2001, Poledne and Zicha 2018). Increased their LDL-cholesterol levels were closely related to the proportion of pro-inflammatory macrophages in the white visceral adipose tissue and it is supposed to be a clear advantage to fight infectious diseases and, consequently longer life (until the 20th century). By contrast, later increased consumption of saturated fatty acids (SAFA) markedly increases their LDL-cholesterol levels and shortens their life expectancy. A definite disadvantage of increased cholesterol levels due to increased cholesterol inflow into the arterial wall is that this process entails pro-inflammatory macrophage polarization (Lemaire-Ewing *et al.* 2012, Poledne *et al.* 2016).

The dietary-induced inflow into the arterial wall works in synergy with pro-inflammatory macrophage polarization (Lemaire-Ewing *et al.* 2012, Poledne *et al.*

2016). Whereas the causality between increased LDL-cholesterol levels and atherogenesis was demonstrated almost half a century ago (a successful decrease in cardiovascular disease (CVD) mortality through statin use), the causality between inflammation and atherogenesis – through effective reduction of myocardial infarction-related mortality with antibody against interleukin 1 β , a key marker of pro-inflammatory status – was demonstrated only recently (Ridker *et al.* 2017) despite initial reports of increased CVD risk of individuals with increased CRP levels (Ridker *et al.* 1997). The interplay between low-grade inflammation and increased cholesterol levels was also nicely documented in the experimental model of apoE KO mice in which circulated monocytes enriched by cholesterol (only after a few days of the Western-type diet) are more likely to cross the arterial endothelium and invade the intima (Foster *et al.* 2015). The situation is more complex in men, it is widely accepted that low-grade inflammation induced by adipose tissue plays the most important role.

Although visceral adiposity was shown to raise the risk of CVD as early as the 1940s (Vague 1947), hard evidence for the pathological potential of adipose tissue macrophages was lacking for decades. Step by step, starting from the newly described metabolic aspect of the role of adipose tissue (in addition to energy storage and adipocyte hypertrophy) (Avogaro *et al.* 1965) through metabolic disease as the direct effect of obesity to the term “metabolic syndrome” (Reaven 1994), adipose tissue is now considered an endocrine organ (Bays 2011). Today, the crucial role of adipose tissue in the development of low-grade inflammation is beyond any doubt. The close interconnection between metabolic and immune dysfunction made researchers coin the term immunometabolism, which is now commonly used to describe obesity-related pathologies of adipose tissue.

Adipose tissue as an immune organ

In addition to adipokine regulating primarily metabolic function, white adipose tissue is also an important source of adipokines implicated in the regulation of immune and inflammatory responses. One of these is resistin produced in humans mostly by adipose tissue macrophages. Resistin has primarily pro-inflammatory activity with an indirect effect on metabolism, mediated through increased expression of pro-inflammatory cytokines, in particular tumor necrosis factor α (TNF α) and IL-6. Another adipokine, visfatin, is

secreted by several cell types including adipocytes, macrophages and endothelial cells and has been shown to play an important role in vascular cell inflammation through the NF- κ B pathway and endothelial adhesion molecule expression (Lee *et al.* 2009). Other primarily pro-inflammatory (retinol-binding protein 4) and anti-inflammatory (omentin-1) adipokines have been discovered and, given the interconnection of metabolic and immune processes, they have also been implicated in the pathogenesis of CVD (Landecheo *et al.* 2019). Besides, adipose tissue produces a large number of cytokines and chemokines that are not tissue-specific and are inseparably involved in the immune response. It is through their secretion that adipose tissue is systematically involved in the activity of an individual's immune system under both physiological and pathological conditions.

The most thoroughly investigated cytokine secreted by adipose tissue is TNF α . The predominant source of this cytokine are macrophages, however, the expression of TNF α in adipose tissue increases with obesity (Hotamisligil *et al.* 1995). This pivotal cytokine stimulates other pro-inflammatory cytokines, mainly IL-6 and IL-1 β (Chen and Goeddel 2002), and also, downregulates adiponectin secretion (Bruun *et al.* 2003). Its role in diet-induced insulin resistance has been conclusively demonstrated and was one of the first proven links between obesity, inflammation and metabolism (Hotamisligil and Spiegelman 1994). TNF α is also involved in atherogenesis as it induces endothelial dysfunction with an obvious link to loss of endothelial protective role (Viridis *et al.* 2019).

In healthy individuals, the participation of adipocyte-derived IL-6 to its systemic levels are estimated to be about one third of total production. Although IL-6 was traditionally believed to possess clearly pro-inflammatory activity and considered a risk factor for the development of type 2 diabetes in humans (Pradhan *et al.* 2001), this view has been recently challenged (Wueest and Konrad 2020).

On the other hand, the anti-inflammatory cytokine interleukin 10 (IL-10) is also secreted by adipose tissue (mainly by regulatory T cells and M2 macrophages) to balance the niveau of local inflammation *in situ* (Juge-Aubry *et al.* 2005), its plasma levels have been suggested as a biomarker of obesity-related metabolic disorders (Kulshrestha *et al.* 2018).

Similarly, a host of other important cytokines have been implicated in adipose tissue inflammation with

their respective roles recently summarized (Unamuno *et al.* 2018).

Focus on Macrophages

Macrophages, the essential components of innate immunity, are one of the most abundant and heterogeneous groups of immune cells, with scavenging of pathogens and damaged cells long regarded as their main function. However, any individual is facing constantly changing threats and macrophages are perfectly adapted to cope with them to maintain the specific tissue environment and to restore physiological state. The original assumption was that monocytes that cross the arterial wall and enter tissues to be transformed to macrophages are the sole source of tissue macrophages. However, this concept was refuted long ago as a proportion of tissue macrophages appears to be deposited in tissues before birth and maintained by self-renewal (Lumeng *et al.* 1972).

Given the broad spectrum of macrophage functions and processes they are involved in, which range from sensing pathogens through removal of cell debris, modulation of inflammation, production of key cytokines to suppression of inflammation in the healing phase, it is not surprising that a highly variable receptor profile with corresponding functional activity exists. Not only are there detectable differences between these tissue-specific macrophages, macrophage subtypes can also be identified within a single type of tissue. Still, there has not been unanimity about the plasticity of fully mature resident macrophages (Lavin *et al.* 2014, van de Laar *et al.* 2016), which is also why it is virtually impossible to comprehensively examine this entire group of cells.

A widely accepted approach is to classify macrophages by the stimuli leading to their differentiation and functional activity (Murray *et al.* 2014). Macrophage subtypes should be perceived as a continuum with diverse patterns of inflammatory and metabolic pathways as changes in tissue microenvironments drive their alteration to participate in restoring homeostasis. This concept, however, may be a simplified one to elucidate macrophage function. On the one hand, M1 phenotype polarization is driven by interferon γ (INF γ) and LPS stimulation and is characterized by high expression of pro-inflammatory cytokines (IL-1 β , TNF α , IL-6) and a high capacity to produce reactive radicals. These characteristics impart the M1 subpopulation potent phagocytic, microbicidal and

tumoricidal activity. By contrast, M2 macrophage polarization is induced by IL-4 and IL-10. M2 macrophages display with a limited capacity to kill pathogens and due to high expression of anti-inflammatory and regulatory cytokines are involved in tissue remodelling, promotion of regenerative processes, formation of the extracellular matrix and also tumor progression.

Phenotypes of adipose tissue macrophages

Macrophages are the most numerous immune cells in stromal vascular fraction and play an important role in both physiological and pathological conditions of adipose tissue. Their numbers increase with obesity, in experimental models they make up 40 % of non-adipocyte cells in adipose tissue (Weisberg *et al.* 2003). The increase in adipose tissue mass associated with increased secretion of adhesion molecules and chemokines (Takahashi *et al.* 2003) results in the migration of immune cells, predominantly monocytes, into adipose tissue. Indeed, the expression of monocyte chemoattractant protein (MCP-1) in adipose tissue preceded metabolic sequels of obesity (Xu *et al.* 2003). While MCP-1 is formed mainly by adipocytes, it is also activated macrophages which contribute to MCP-1 production (as a result of interaction of SAFA with Toll-like receptor (TLR) 4 (see below). This feedback stimulates macrophage infiltration and acceleration of inflammation in adipose tissue (Bai and Sun 2015). The final number of macrophages results not only from their entry into adipose tissue, local proliferation, apoptosis and differentiation from pre-adipocytes (Charrière *et al.* 2003) but, presumably, also from macrophage outflow from tissues (Amano *et al.* 2014, Ramkhalawon *et al.* 2014).

Originally, macrophage accumulation was considered a marker of pro-inflammatory status of adipose tissue, however, as mentioned above, the macrophage population is highly variable and may evoke different responses. In physiological, non-obese adipose tissue, macrophages tend to polarize – under the influence of the so-called Th2 cellular response (characterized by the production of typical cytokines such as IL-4, IL-5 and IL-13) – predominantly towards the M2 phenotype. These M2 macrophages of physiological adipose tissue express arginase, inhibit iNOS activity, and produce anti-inflammatory cytokines such as IL-10, which maintain adipocyte insulin sensitivity by inhibiting TNF α

(Smallie *et al.* 2010) thus significantly affecting the local microenvironment and adipocyte function. The metabolic demands of M2 macrophages are provided with long-term sustainable energy sources mainly through fatty acid oxidation, tricarboxylic acid cycle and mitochondrial respiration. The preferential energy substrate of M2 macrophages is oxidative phosphorylation. However, excess of M2 macrophages is also associated with aberrant fibrogenesis (Hou *et al.* 2018), which may hinder adipose tissue remodelling in obesity. The pro-inflammatory environment in obese adipose tissue leads to rearrangement toward prevalence of M1 macrophages (Thomas and Apovian 2017). Metabolic brake in tricarboxylic acid cycle (Jha *et al.* 2015) was identified in these proinflammatory macrophages resulting in a shift toward increased glucose anaerobic glycolysis as a key source of energy even under aerobic conditions. This M1 macrophage predominance results in the disruption of the functional state of adipocytes (Jager *et al.* 2007, Permana *et al.* 2006) reducing insulin sensitivity already in clinically healthy overweight individuals (Rydén *et al.* 2019). It is the ratio of diverse adipose tissue macrophage subtypes which determines the inflammatory status of adipose tissue. Furthermore, although the concept of M1 pro-inflammatory and M2 anti-inflammatory macrophages is accepted for adipose tissue macrophages (ATM), their phenotypic determination is very uncertain and differences between men and animal models exist. The above facts likely contribute to significant contradictions in published data, where pro-inflammatory characteristics of adipose tissue such as obesity were associated with the expression of anti-inflammatory markers (Fjeldborg *et al.* 2014, Zeyda *et al.* 2007), in addition, macrophages both expressing pro- and anti-inflammatory phenotypic or functional features have also been reported (Bourlier *et al.* 2008, Zeyda *et al.* 2007).

The relationship between adipocytes and macrophages appears to be reciprocal, as paracrine interactions between adipocytes and macrophages have been demonstrated, in which free fatty acids (FFA) and TNF α appear to play a key role (Suganami *et al.* 2005). TNF α release from pro-inflammatory macrophages trigger inflammatory changes in adipocytes. These changes include not only FFA release that aggravates inflammation in adipocytes but, also, MCP-1 secretion entailing further infiltration of monocytes from the bloodstream. It has also been recently documented in an *in vitro* model that the interaction of macrophages and

adipocytes led to an increase in the production of oxygen radicals in macrophages, with triglyceride content in adipocytes increasing by more than 70 % (Nimri *et al.* 2019), again confirming the possibility of significant paracrine interaction of these cells in fat *in situ*. The intercellular environment in adipose tissue is rich in glucose, insulin, palmitate and chemically (oxidation and glycation) modified metabolites and therefore several other tissue-specific macrophage phenotypes with unique properties have been identified in adipose tissues (Li *et al.* 2020). Several metabolic pathways have been directly linked to macrophage polarization, giving rise to metabolically activated macrophages (MAM). Saturated fatty acids have been shown to stimulate TLR4 on macrophages and adipocytes (McKernan *et al.* 2020) and to activate NF- κ B (Rocha *et al.* 2016). Lipolysis of triglycerides making up the intracellular content of FFA in adipocytes and consequently their release. An increased intracellular content of saturated FFA (e.g., palmitic acid) leads to changes in the endoplasmic reticulum (similar to other plasma membranes), referred to as endoplasmic reticulum stress (ERS) and subsequent activation of significant pro-inflammatory pathways (associated with activation of c-Jun-N terminal kinase and NF- κ B) (Hotamisligil 2010). According to published data, MAM can be characterized by high expression of ATP-binding cassette transporter A1, perilipin-2 and CD36. A direct effect of environment, which resembles that of adipose tissue, on macrophage polarization was clearly shown (Kratz *et al.* 2014) highlighting the importance of CD36. The CD36 receptor is a scavenger receptor with recognized proatherogenic effects (Park 2014). CD36 expression appears to be regulated at the level of transcription, posttranscriptional modifications (miRNAs) and other processes (Tian *et al.* 2020). Including this marker we were able to identify specific macrophage subpopulation with close relationship to main risk factor of atherosclerosis (Kralova Lesna *et al.* 2018). Our results confirmed the link between adipose tissue macrophages, CD36 overexpression and hypercholesterolemia (Poledne *et al.* 2016) on the one hand, and the fatty acid spectrum in adipose tissue membranes on the other (Poledne *et al.* 2019). It is clear that the fatty acids released from hypertrophied adipocytes also serve as ligands for TLR4 and contribute to further deterioration of physiological regulation (Suganami *et al.* 2005). Moreover, hypertrophied adipocytes have been shown to increase the expression of arachidonic acid converting enzymes to pro-inflammatory

mediators (Mothe-Satney *et al.* 2012). The size of macrophages compared to that of hypertrophied adipocytes renders their phagocytosis impossible and the crown-like structures consisting of several macrophages surrounding the adipocyte with extracellular lysosomal compartments represent the typical histological pattern of obese adipose tissue. These and other processes are involved in the development of pro-inflammatory status of adipose tissue associated with its dysfunction. However, a double-faced feature that includes the beneficial role of MAM in diet-induced obesity has been proposed based on their involvement in dead adipocyte clearance (Coats *et al.* 2017).

Oxidation and glycation of lipoproteins change their properties. These modified particles are perceived by macrophages as what is referred to as danger-associated molecular patterns (through TLR and CD36) activating the inflammatory pathways and macrophage differentiation (Seo *et al.* 2015) and giving rise to antioxidant macrophages (Mox). These Mox, which can be differentiated from both M1 and M2 macrophages by treatment with oxidized lipids, are characterized by upregulation of antioxidant enzymes and reduced phagocytic and chemotactic capacity (Kadl *et al.* 2010).

Another macrophage phenotype identified in adipose tissue are iron-rich macrophages, which are characterized by an anti-inflammatory and iron-recycling gene expression profile (Orr *et al.* 2014). These authors demonstrated, *in vivo*, obesity-evoked reduction of the iron content of specific adipose tissue macrophages and, also, reduction of the gene expression of iron importers in these macrophages.

A specific feature of M2 macrophages in white adipose tissue may also be their production of catecholamines and the induction of changes referred to as browning, i.e. the expression of uncoupling protein-1 (Villarroya *et al.* 2018). Although the important role of M2 macrophages in this process has been repeatedly confirmed (Nguyen *et al.* 2011, Qiu *et al.* 2014, Rajasekaran *et al.* 2019), other authors have questioned it (Fischer *et al.* 2017).

Adipose tissue cell membrane composition and inflammation

The potential of dietary fats to influence systemic inflammation has been supported by numerous published data (see review Calder 2017). To give an example – in a well-controlled experiment, 15 healthy female volunteers (peri- and postmenopausal) and employees of our Institute were included in a randomized double crossover study (Kralova Lesna *et al.* 2013). The participants were supplied, over a period of 3 weeks, a diet either rich in SAFA or polyunsaturated fatty acids (PUFA) (40 % energy of total fat) with a wash-out period in between. A comparison of the final datasets of both dietary regimens revealed an increase in LDL cholesterol levels (after lipoprotein fraction isolation by ultracentrifugation) by almost 1 mmol after the SAFA diet. Also, HDL cholesterol levels increased significantly after SAFA compared to the PUFA diet (Kralova Lesna *et al.* 2013). In addition to lipoprotein changes, the SAFA diet also induced systemic inflammation assessed by high-sensitive CRP.

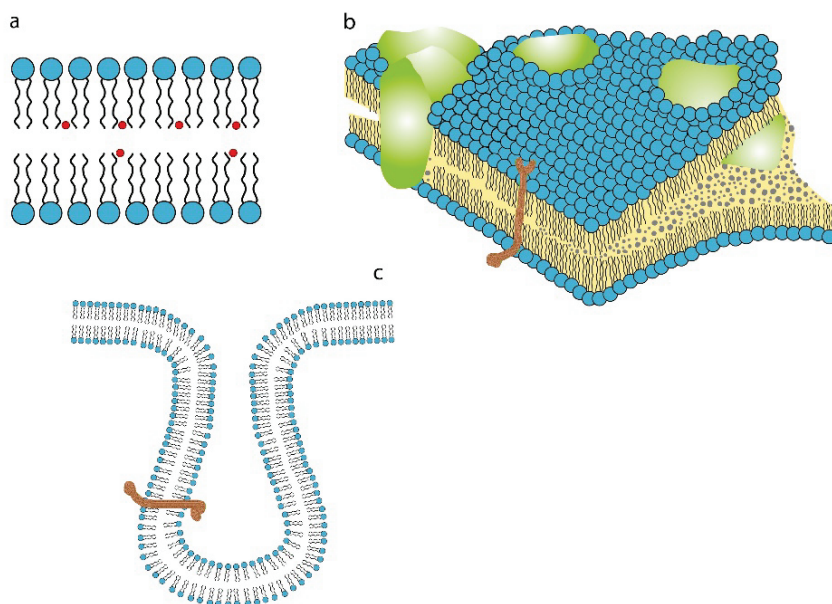


Fig. 1. a) Plasma membrane phospholipid (and cholesterol - red circle) structure of the outer and inner parts. b) Cell surface covered and uncovered by the raft (green parts). Transmembrane position of the specific receptor (brown). c) Specific receptor and its location in the coated pits.

It has been documented recently that the cell membrane structure is related to the pro-inflammatory activity of adipose tissue (Poledne *et al.* 2019). After four decades of systematic research, the exact structure of the plasma membrane remains poorly understood. The structure and dynamics of plasma membrane have been analyzed using physicochemical techniques (Devaux and Hermann 2012). The cell membrane is a semipermeable bilayer that consist mainly of phospholipids enveloping the cell and delimiting cytoplasm and its intracellular organelles from the cell surroundings. The cell membrane proteins are attached loosely within the membrane and are allowed to diffuse laterally or to rotate about. Signal from the extracellular space to cytosol (and back) has been well described, but much uncertainty remains over the role of a surface lipid raft (Simons and Gerl 2010). A lipid raft is defined as a liquid-moving cholesterol-sphingolipid and protein enriched domain compartmentalizing cellular processes (Pike 2006), Fig 1. However, lipid rafts relevance and even existence remains elusive, because no method is currently available to document its presence on the surface of living cells (Levental *et al.* 2020). It has been conclusively established that lipid rafts are only present in the parts of the cell membrane where important cellular processes take place (Levental *et al.* 2020). Still, an effect of some substances on membrane function has been demonstrated. Specifically, the caveolin proteins bind tightly to cholesterol in the cell membrane affecting its function (Parton and Simons 2007) whereas the palmitoleate molecule, which is surprisingly plentiful in the cell membrane, substantially modulates the inflammatory response (Medina *et al.* 2006). The important role of palmitoleate in the stimulation of pro-inflammatory polarization of adipose tissue macrophages was recently documented (Poledne *et al.* 2019), yet its exact mechanism remains unknown. However, the majority of studies have logically focused on the effect of cholesterol within the plasma membrane and its function.

It is of interest that, while even markers of low-grade inflammation (CRP, TNF α , Il-18) may disappear after successful weight loss, adipose tissue infiltration with macrophages will persist (Ara *et al.* 2020).

Cholesterol and the plasma membrane

The cholesterol molecule within the plasma membrane has been shown to play a most important role

in various cell functions (Varshney *et al.* 2016). For example, cholesterol enrichment of the plasma membrane results in its lower fluidity which may, in turn, prompt the signaling pathway to promote the formation of some solid tumors such as breast and prostate cancer (Hryniewicz-Jankowska *et al.* 2019). An increase in cholesterol content in the cell membrane will enhance the pro-inflammatory effect of macrophages through the TLR's system (but not only through TLR4).

The reported data conclusively documenting the close correlation between the levels of intravascular cholesterol and inflammation confirm the bi-directional effect of cholesterol molecules and pro-inflammatory macrophages, as suggested by both various experimental models and tissue culture (Tall and Yvan-Charvet 2015, Yvan-Charvet *et al.* 2008) and (Medbury *et al.* 2015), respectively. This close relationship of non-HDL cholesterol and pro-inflammatory status of human visceral adipose tissue is in line with the summarized data on cholesterol and inflammation in experimental models fed a high-fat, high-cholesterol diet (Tall and Yvan-Charvet 2015).

Cholesterol enrichment of the plasma membrane as a consequence of an increase in plasma cholesterol levels has been implicated in adipose tissue dysfunction (Aguilar and Fernandez 2014), local systemic inflammation (Busnelli *et al.* 2013) and pro-inflammatory macrophage polarization in adipose tissue. The plasma levels of both non-HDL cholesterol and LDL cholesterol have been shown to correlate with the proportion of pro-inflammatory macrophages in human adipose tissue (Poledne *et al.* 2016). While cholesterol exchange between lipoproteins and macrophage surface has not been demonstrated to date, it was recently shown for red blood cells (Lai *et al.* 2019). On the other hand, an inverse correlation between alternatively polarized macrophages and non-HDL cholesterol levels has also been reported (Poledne *et al.* 2016).

Importantly, cholesterol density within the cell membrane is not distributed homogeneously. Caveolae are enriched by cholesterol compared to the neighboring parts of the membrane (Ortegren *et al.* 2004). Cholesterol depletion in the caveolae may completely destruct their function (Rothberg *et al.* 1992). The effects of diet on low-grade inflammation can be explained by plasma membrane enrichment with the cholesterol molecule (Scalia, 2013).

Given the above, one may conclude that lipids – and the cholesterol molecule in particular – play a most

important role in cell membrane function (Simons and Gerl 2010). The so-called “pleiotropic effects of statins” may be therefore explained by cholesterol lowering which, in turn, slows cholesterol transport to the lipid raft thus affecting macrophage polarization. An increase in the mole percentage of cholesterol and different proportions of saturated and ω 3 PUFA in the lipid raft may be able to change the caveolae of circulating monocytes and their ability to enter the arterial wall and to mature into residential macrophages. Cholesterol molecules play a multifunctional role in atherogenesis (Pirillo *et al.* 2018) not only based on to their increased levels in circulating LDL particles but, also, in the immune response based on signal transduction in inflammation (Lemaire-Ewing *et al.* 2012).

Effect of fatty acid composition of the plasma membrane

Just another and most interesting aspect of adipose tissue inflammation are the different proportions of palmitate and ω 3 fatty acids in the plasma membrane. Evidence has been mounting over the last decade that locally increasing SAFA levels could be directly related to adipose tissue pro-inflammatory status (Siri-Tarino *et al.* 2010). Palmitate has been shown to enhance the pro-inflammatory activity in adipocyte tissue culture (Ajuwon and Spurlock 2005) through NF- κ B and substantially increase IL-6 production. A similar effect was documented in human macrophage culture (Laine *et al.* 2007) where laurate, palmitate, and stearate stimulated NF- κ B activation, whereas ω 3 PUFA were able to inhibit it. It is not clear yet whether this pro-inflammatory effect of SAFA is produced by palmitate itself or is the result of palmitoleate (desaturation product of palmitate by stearyl-CoA desaturase). The highly elegant methodological approach proposed by Suganami (Suganami *et al.* 2007), who used a co-culture of adipocytes and macrophages, demonstrated that NF- κ B activation was much greater compared with seen with separate cultures of both cell types serving as controls. The interplay between adipocytes and macrophages in co-culture activated the NF- κ B pathway in macrophages increasing TNF α production which, in turn, induced adipocyte lipolysis and SAFA release. The endogenous free fatty acids (predominantly palmitate) released from co-cultured adipocytes were able to activate the NF- κ B pathway in macrophages and, possibly, increase TNF α production. Quite surprisingly, the adverse effects of

SAFA on the innate immune response may be inhibited by high-density lipoproteins (Zhang *et al.* 2017). Mechanisms whereby SAFA activate the regulatory role of Toll-like receptor 4 include their numbers, protein dimerization, and changes in lipid rafts. All of these three mechanisms have been shown to enhance the inflammatory effects of SAFA (Lee and Hwang 2006).

The proportions of saturated and unsaturated fatty acids (mainly ω 3 PUFA) in cell membrane phospholipids have been found to be responsible for inflammatory changes. Studies with high intake of fish oil tested the hypothesis that the established effects of ω 3 eicosapentaenoic fatty acid (EPA) could afford protection against CVD (Calder *et al.* 2002). However, such a protective effect has not been conclusively confirmed as, while some studies reported a beneficial effect in terms of coronary heart disease prevention, the outcomes of other studies were just the opposite. The effect of fish oil has been shown to depend on the quality of fish oil products. The negative results of dietary intervention may be attributable to increased contents of oxidized fish oil and increased proportions of SAFA in dietary supplements (Manson *et al.* 2019, Mason *et al.* 2020). Use of fish oil capsules originally employed to treat high plasma triglyceride level brought very inconsistent results (as reported earlier). On the contrary, in the Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial (REDUCE-IT), pure eicosapentaenoic acid administration resulted in triglyceride lowering (Bhatt 2019). An additional 25 % reduction in ischemic heart disease has been documented in patients with residual risk (while already on statin treatment) in this trial. There are several possibilities to explain this successful effect of a pure molecule. Among these, an ω 3 fatty acids induced decrease in monocyte adhesion or antithrombotic effect are plausible, besides, an effect of ω 3 FA incorporation to the plasma membrane leading to a decrease in the proportion of pro-inflammatory macrophages has also been demonstrated (Poledne *et al.* 2019).

Increased consumption of dietary EPA results in their accelerated incorporation into plasma membranes of monocytes and macrophages (Calder *et al.* 2002) and, eventually, inflammation and production of cytokines decreasing monocyte and macrophage adhesiveness to the endothelium (Mason *et al.* 2020). The result is less substrate for eicosanoid synthesis from arachidonic acid known to promote inflammation.

Unlike α -linolenic and eicosapentaenoic acids, the SAFA in the plasma membrane are associated with

increased adipose tissue macrophage polarization to pro-inflammatory status (Poledne *et al.* 2019) likely due to reduced plasma membrane fluidity (Dunbar *et al.* 2015). Increased proportions of SAFA in the plasma membranes in adipose tissue was shown to increase the proportion of pro-inflammatory macrophages in human visceral adipose tissue (Poledne *et al.* 2019).

Part of the data reported above were obtained by analysis of adipose tissue in various animal models. It is always difficult, if not impossible, to extrapolate experimental data from animal models to human pathology. For example, the issue of macrophage specification is even more complicated as similarities between human (CD14, CD16 or CD163) and mice (CCR5, Cx3CR1) surface receptor function are only assumed and difficult to establish. Given the above, no clear-cut definition of the pro-inflammatory M1 and anti-inflammatory M2 phenotypes has been proposed and their respective roles in atherogenesis and inflammation remain poorly understood (Calder 2017).

The pro-inflammatory effect of free fatty acids in adipose tissue

The relationship between adipose tissue and atherosclerosis was explained by the “sick fat” concept proposed in the early 21st century (Bays 2011) based on the unprecedented increase in the incidence of obesity during the last decades of the 20th century. An important passive role of adipose tissue lasted 100 000 years, when it served as a reserve metabolic source of energy for man during frequent periods of fasting or even starvation due to extremely limited energy availability. This situation changed dramatically during the last century. Due to easy access to food for most of the population around the world, amount of body fat increased with the increasing prevalence of obesity. This applies not only to industrialized nations but, also, to most regions the developing world. The real fasting period during the day practically disappeared in most people.

With widely available energy, lipolysis induced by cyclic ATP continues to deliver FFA to other tissues despite adequate energy supply from the intestine. In the absence of adequate amount of energy produced in the liver or muscle tissue, these FFA increase their plasma levels. Chronically elevated FFA levels impair insulin sensitivity (Boden and Shulman 2002) and their return to adipose tissue. Almost a third of the plasma FFA undergoes re-esterification (Nye *et al.* 2008) to

triglycerides within adipose tissue in the process requiring additional energy supply (Izumi 2012). The remaining FFA are trapped in other organs increasing triglyceride levels in the muscle, liver and even pancreas. There are data documenting triglyceride accumulation in Langerhans cells, which triggers insufficient insulin production. Similarly, triglycerides tend to accumulate in the liver leading to the development of non- alcoholic liver steatohepatitis (NASH) (Cusi 2009).

A more detrimental process was documented in adipose tissue residential macrophages (Wong *et al.* 2019) where the FFA stimulate their surface Toll-like receptor (Wong *et al.* 2019) setting off an intracellular cascade leading to NF- κ B activation in cytosol, promoting inflammation and production of cytokines in the nucleus (IL-6, TNF α , IL-1 β , and others) (Blüher 2016, Wang *et al.* 2017). Consequently, adipose tissue becomes more increasingly infiltrated by macrophages (Moreno-Indias *et al.* 2016), even in individuals with normal body weight. Adding further complexity to the picture, unsaturated FFA inhibit the saturated FFA-induced pro-inflammatory effect (Bernardi *et al.* 2018).

It is of interest that, while even markers of low-grade inflammation (CRP, TNF α , IL-18) may disappear after successful weight loss, adipose tissue infiltration with macrophages will persist (Ara *et al.* 2020).

Conclusions

The risk of ectopic fat involvement in the development of cardiovascular disease was documented more than half a century ago. Nowadays we understand that the negative effect of enlarged adipose tissue occurs mainly through inflammation, with adipose tissue macrophages playing a pivotal role in this process. FFAs released from adipocytes activate adipose tissue macrophages through TLR, NF- κ B stimulation, and pro-inflammatory cytokine secretion driving the pro-inflammatory status of the whole body. The causality of the relationship of the pro-inflammatory status to atherosclerosis development was proved using human monoclonal antibodies targeting IL-1 β . The composition of the plasma membrane of adipose tissue macrophages serves as a playground in which signal proteins activate inflammation. Increased cholesterol content in the plasma membrane resulting from the interaction with pathologically increased plasma lipoproteins stimulates the pro-inflammatory polarization of macrophages. This

similar effect is driven by increased proportions of palmitate and palmitoleate in cell membrane phospholipids. On the other hand, increased proportions of α -linolenic acid and other ω 3 fatty acids in plasma membrane phospholipids decrease the proportion of pro-inflammatory macrophages in adipose tissue. As different role of SAFA and PUFA is similar to their roles in the regulation of specific LDL receptors and, consequently, plasma LDL cholesterol levels, it is intriguing to speculate that these processes act together. It supports the hypothesis of the complex effect of the replacement of

animal fat by vegetable fat, where lowering plasma LDL-cholesterol levels and decreasing pro-inflammatory status works in synergy.

Conflict of Interest

There is no conflict of interest.

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