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Review

Obesity, Inflammation, Toll-like Receptor 4 and Fatty Acids

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Abstract: Obesity leads to an inflammatory condition directly involved in the etiology of cardiovascular diseases, type 2 diabetes mellitus and certain types of cancer. The classic inflammatory response is an acute reaction to infections or to tissue injuries, and it tends to move towards resolution and homeostasis. However, the inflammatory process observed in individuals affected by obesity and metabolic syndrome differs from the classical inflammatory response in certain respects. This inflammatory process manifests itself systemically and is characterized by a chronic low-intensity reaction. The toll-like receptor (TLR) 4 signaling pathway is acknowledged as one of the main triggers of the obesity-induced inflammatory response. The aim of the present review is to describe the role played by the TLR4 signaling pathway in the inflammatory response and its modulation by saturated and omega-3 polyunsaturated fatty acids. Studies indicate that saturated fatty acids can induce inflammation by activating the TLR4 signaling pathway. Conversely, omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, exert anti-inflammatory actions through attenuation of the activation of the TLR4 signaling pathway by either lipopolysaccharides or saturated fatty acids.

Keywords: inflammation; Toll-like receptor 4; obesity; fatty acids

1. Obesity

Obesity is a multifactorial and polygenic condition that has become a very concerning public health issue affecting both developed and developing countries [1-3]. Overweight individuals (defined as body mass index [BMI] ≥ 25 kg/m²) account for approximately 30% of the global population, i.e., 2.1 billion people, of whom more than 600,000 are classified as obese (defined as BMI ≥ 30 kg/m²) [4]. The analysis conducted by the *Global Burden of Disease Study 2013* showed that the overweight prevalence increased to 27.5% of adults and 47.1% of children in the past three decades [5]. The prevalence of obesity is currently higher in developed countries; nevertheless, approximately two-thirds of the obese population lives in developing countries [6]. Based on the current scenario, it is estimated that up to 50% of the global population will be classified as overweight or obese by 2030 [7]. Approximately 35% of adult individuals and 17% of children and

adolescents (2 to 19 years old) are considered obese (defined by values above the 95th percentile of the BMI curve of these age groups) in the United States. It is estimated that approximately 300,000 people die due to obesity in the U.S. every year, which is the second highest cause of preventable death [8].

Cardiovascular diseases, type 2 diabetes (DM2), non-alcoholic fatty liver disease and cancer stand out among the main health issues responsible for morbidity related to the obesity [9]. Obesity treatment and the treatment of its associated complications in developing countries has led to significant cost increases in healthcare. Costs linked to DM2, in particular, stand out, since 20%–30% of overweight people present with a DM2 diagnosis, while 85% of diabetic patients are overweight or obese [10]. Calle et al. [11] conducted a prospective study of more than one million men and women and found that the lowest mortality rates, for all causes, in both men and women, occur in individuals with BMIs between 23.5 and 24.9 and 22.00 and 23.4 kg/m^2, respectively. Another study including 900,000 adult individuals found that BMIs above 25 kg/m^2 were associated with a 30% increase in general mortality rate per each 5 kg/m^2 increase [12].

Obesity results from the interactions of different factors, including genetic, metabolic, behavioral and environmental ones. Accordingly, the dramatic increase in obesity prevalence rates suggests that behavioral and environmental components are the main factors responsible for obesity, with an emphasis on eating habits and exercise. With regard to eating, modern societies converge to an eating pattern called the Western diet, which is characterized by the intake of foods with high energy densities. Such densities derive from the high contents of fat and carbohydrate, especially sugars, found in these food types, a fact that contributes to obesity development [13,14].

The profile of fatty acids present in a diet may also be relevant to obesity. It is worth highlighting that, according to anthropological and epidemiological studies, humans from the Paleolithic Era—40,000 years ago—consumed a ratio of omega-6 (ω-6) to omega-3 (ω-3) polyunsaturated fatty acids of approximately 1, mainly due to a high intake of marine and vegetable sources of ω-3 polyunsaturated fatty acids (PUFAs). However, there was a significant increase in the intake of lipids, *trans* fatty acids and ω-6 PUFAs after the Industrial Revolution, as well as a small increase in the intake of ω-3 fatty acids; meanwhile, intakes of vitamins C and E decreased. Such changes are particularly relevant if one takes into account the participation of these nutrients in the inflammatory response, which is linked to the physiopathology of different non-transmissible chronic diseases, such as obesity, DM2, cardiovascular diseases, hypertension and cancer [15-17].

2. Inflammation, Adipose Tissue and Obesity

Inflammation is a central component of innate immunity, and microorganism destruction is the prime function of the inflammatory response, a process that involves the participation of effector cells in contact with pathogens living in the infected tissue. Microbial components, such as lipopolysaccharides (LPS) found in the cell wall of Gram negative bacteria, can trigger an inflammatory response through their interactions with cell-surface receptors found, for instance, in cells from the immune system, such as macrophages and neutrophils. Inflammation in response to microorganisms involves increased synthesis and secretion of a number of mediators, including chemokines and cytokines. The latter include tumor necrosis factor (TNF)-α and interleukin (IL)-1

which act on endothelial cells and leukocytes to promote the recruitment and activation of leukocytes in the inflammatory area [18,19].

Inflammation can be classified as acute or chronic. Acute inflammation presents via three principal components: (i) changes in the vascular caliber, which result in increased blood flow in the inflammatory focus; (ii) structural changes in the microcirculation, which favor the exit of plasma proteins and leukocytes from the blood to the tissue; and (iii) adhesion and transmigration of leukocytes from the microcirculation to the tissue as well as their further activation, which allows the elimination of harmful agents. As soon as the infection is eliminated, or at least controlled, mechanisms are activated that act to limit any type of aggression against the host and to initiate the tissue repair process. Such a process aims to reduce the inflammation and is termed resolution. Resolution is now known to be an active process involving the activation of negative feedback mechanisms, such as anti-inflammatory cytokine secretion, a reduction in receptor expression, activation of regulatory cells, and production of pro-resolving lipid mediators [20-22].

Histamine, bradykinin, neuropeptides, prostaglandins, thromboxanes, leukotrienes and platelet-activating factor stand out among the non-cytokine/chemokine mediators involved in the inflammatory response. The generation of eicosanoids initially occurs due to activation of phospholipase A2, which hydrolyzes membrane phospholipids to yield a free fatty acid. Arachidonic acid, an ω-6 PUFA, is predominant among the fatty acids released by phospholipase A2. The released fatty acids are used as a substrate by the cyclooxygenase enzymes (COX), which catalyze the synthesis of prostaglandins and thromboxanes, as well as by lipoxygenase (LOX) enzymes, which catalyzes the synthesis of leukotrienes. Such mediators are responsible for many aspects of the inflammatory response, such as vasodilation (prostaglandin E_2) and leukocyte migration (leukotriene B_4) [23-25].

Chronic inflammation involves progressive changes in inflammatory cells as well as in tissue destruction and repair due to the on-going inflammatory process. Accordingly, inflammation can become pathologic because of the loss of tolerance or regulatory processes. As a result, there is an increase the plasma concentrations of many inflammatory biomarkers and in the number of activated inflammatory cells in the bloodstream as well as in the primary lesion area. Such changes can be easily observed, for instance, in patients with frank chronic conditions like rheumatoid arthritis and inflammatory bowel diseases [26,27].

Chronic inflammation can also present at lower intensities than seen in the classic inflammatory diseases. Evidence that obesity results in inflammation started emerging in the 1990s. This inflammation is directly involved in the etiology of cardiovascular diseases, DM2 and certain cancer types [28]. Hotamisligil et al. [29] found that genetically obese rodents, such as db/db and ob/ob mice and fa/fa rats, had increased expression of the TNF-α gene in white adipose tissue. They identified that neutralization of TNF by anti-TNF-α antibodies mitigated the resistance of these animals to insulin action, establishing a link between inflammation, insulin resistance and hyperglycemia. Macrophages from the stromal vascular fraction of adipose tissue appear to be the main cell type responsible for TNF-α and IL-6 release from the adipose tissue. The increased concentration of cytokines in this tissue is mostly derived from the infiltration of M1 macrophages, which are activated in the classical way and characterized by the high expression of pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 [30-32] (Figure 1). It should be noted that macrophages correspond to about 40% of total white adipose tissue cells in obese mice and humans,

133 compared to only 18% in lean controls [33]. In the white adipose tissue, the expression of monocyte
134 chemoattractant protein (MCP)-1 correlates positively with adiposity, and is also higher in visceral
135 adipose tissue compared to subcutaneous adipose tissue [34,35]. The receptor for MCP-1, CCR2 (CC
136 receptor 2), is expressed on monocytes present in peripheral blood and on adipose tissue
137 macrophages. This implies that obesity favors the process of migration of blood monocytes into the
138 visceral adipose tissue of obese individuals, which then differentiate into macrophages. This process
139 is regulated by colony stimulating factors such as macrophage-specific growth factor called CSF -1
140 or M-CSF [36].

141 In mammals, there are two types of adipose tissue: white and brown adipose tissue (BAT). BAT
142 is specialized in the production of heat (thermogenesis) and, therefore, actively participates in the
143 regulation of body temperature. BAT deposits are found in fetuses and newborns. In adult humans,
144 there is a small volume of BAT in the cervical supra-clavicular, supra-adrenal and para-spinal
145 regions [37,38]. Brown and white adipocytes appear to have different physiology and opposing
146 functions [39] Beiging/browning of white adipose tissue promotes energy expenditure by triggering
147 thermogenesis, which suppresses diet-induced weight gain, as well as enhancing the efficiency of
148 BAT activity [40]. In this context, individuals with low amounts of BAT would be prone to the
149 development of obesity. Studies in animals lacking BAT or uncoupling protein 1 (UCP1) have clearly
150 demonstrated the involvement of BAT thermogenesis in protection against diet-induced obesity (DIO)
151 [41]. Decreasing BAT activity or removal of BAT in mice provokes increased glycemia and plasma
152 triglyceride concentration and promotes insulin resistance [42]. Also, in humans, BAT activity was
153 found to be inversely related to BMI and fat mass [43]. Furthermore, visceral adipose tissue
154 inflammation may also be linked to the lower BAT volume since TNF-α has been shown to induce
155 brown adipocyte apoptosis and hamper BAT differentiation [44].

156 Obesity is a relevant causal factor in the etiology of insulin-action resistance. Thus, obese
157 patients present with reduced insulin action in the skeletal muscle due to lower phosphorylation of
158 the tyrosine residues of the insulin receptor substrate (IRS)-1 and the reduced
159 phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) activity in this tissue. Such an outcome can
160 cause a further reduction in insulin-induced glucose transport into the muscle tissue [45].
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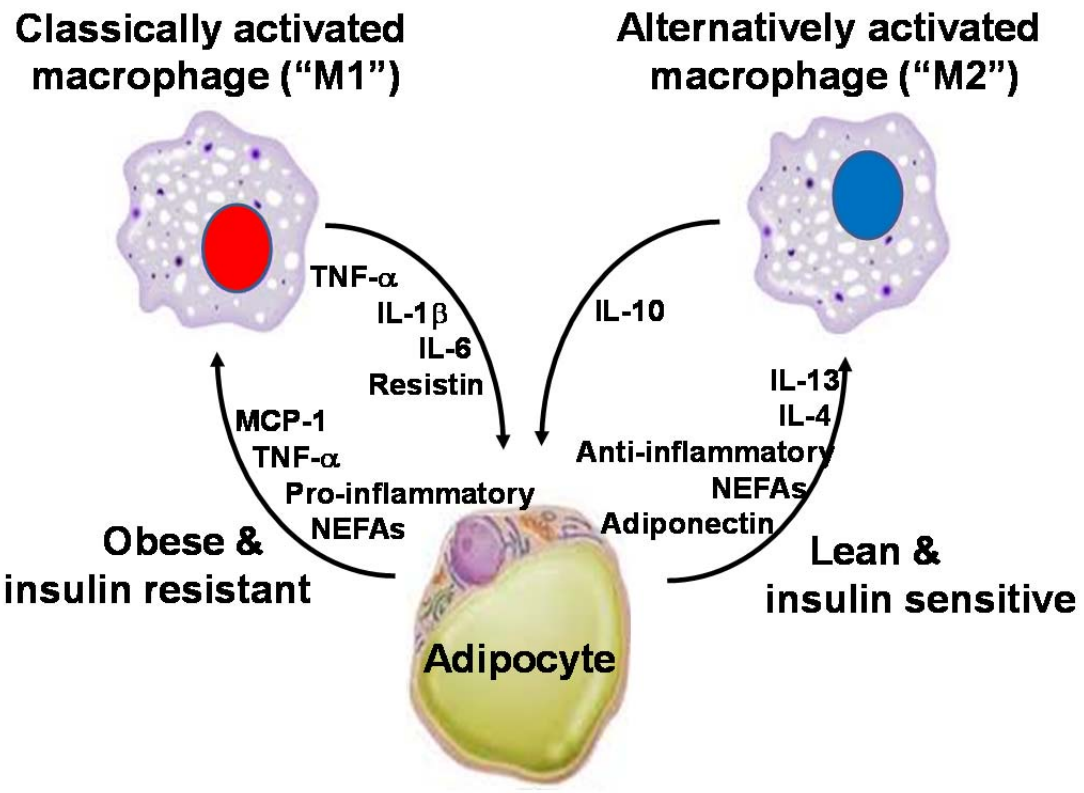


Figure 1. Interaction between M1 and M2 macrophages and adipocytes. Abbreviations: IL, interleukin; MCP, monocyte chemotactic protein; NEFAs, non-esterified fatty acids; TNF, tumor necrosis factor.

An increased inflammatory response is an important factor in the etiology of insulin-action resistance in obese patients. Such a response triggers the activation of protein kinases related to Toll signaling pathways and TNF-α receptors, such as the inhibitor of kappa B kinase (IKK) and c-jun N-terminal kinase (JNK)-1, which are capable of phosphorylating IRS-1 at the serine 307 residue. This reduces IRS-1 interaction with the insulin receptor beta subunit and, consequently, causes decreased insulin signal transduction [46,47]. JNK knockout mice show lower adiposity, enhanced sensitivity to insulin and an increased capacity for insulin receptor signaling even when they are fed a lipid-rich feed. These findings suggest that activation through JNK is an important mechanism linked to insulin resistance in obese patients [48].

Among the inflammatory biomarkers related to obesity, IL-6 favors insulin-action resistance in obese individuals due to the induction of the cytokine signaling suppressor protein 3 (SOCS3), which physically associates itself with tyrosine phosphorylated proteins, such as the insulin receptor. In addition, SOCS3 decreases the phosphorylation of IRS-1 tyrosine, which weakens the IRS-1 coupling to the insulin receptor and the subsequent association between IRS-1 and PI3K. These findings suggest that SOCS3 is a relevant inhibitor of the insulin signaling pathway, as well as allowing a better understanding of the IL-6 effect on the insulin-action resistance induced by obesity [49].

Understanding that the immune system and different metabolic pathways are closely related to each other as well as that they are functionally dependent is essential for studies focused on obesity and on its possible metabolic repercussions. Thus, signaling pathways responsive to

188 nutrient intake and the presence of pathogens are evolutionarily conserved and greatly integrated
189 [50]. The excessive intake of obesity-associated nutrients can be detected by innate recognition
190 receptors, and this results in the activation of pro-inflammatory signaling pathways as well as in
191 stress responses in many parts of the body. This causes low-intensity chronic inflammation, defined
192 by Hotamisligil et al. [30] as metabolic inflammation or as meta-inflammation, which is different
193 from the classic inflammatory response. Moreover, the genesis of this inflammation is closely related
194 to lifestyle and mainly to the quality of diet and exercise [51].

195 Meta-inflammation development is associated with a wide and integrated network of
196 intracellular signal pathways, among which IKK-β and JNK-1 stand out. These proteins induce the
197 synthesis of inflammatory mediators in different cell types. IKK-β and JNK-1 activation results in
198 activating the transcription factors nuclear factor kappa B (NF-κB) and activating protein (AP)-1,
199 which translocate to the cell nucleus and activate the transcription of many genes encoding the
200 proteins involved in inflammation including TNF-α and COX-2. This process allows the continuity
201 of the inflammatory reaction, which is associated with conditions such as atherogenesis and
202 insulin-action resistance [52,53].

203 This systemic inflammatory response mainly originates from adipose tissue, which
204 produces a wide variety of pro-inflammatory cytokines and chemokines called adipokines [23].
205 However, currently, it is known that there are other tissues involved in meta-inflammation, such as
206 the liver [54], pancreas [55], hypothalamus [56,57] and skeletal muscle [58]. It seems likely that the
207 chronic low-grade inflammation that develops in adipose tissue with obesity is "transferred" to
208 these other tissues through the appearance of active inflammatory mediators in the bloodstream.

209 In the context of inflammation and obesity, the role of gut microbiota in the development of
210 metabolic disease should be noted. Studies have shown that certain bacteria populations produce
211 enzymes that increase the efficiency of nutrient digestion, leading to improved nutrient supply to
212 the host, therefore, contributing to increased energy storage in the adipose tissue. The resulting
213 increase in body adiposity can trigger the development of insulin resistance. There is also evidence
214 that the gut microbiome can modulate genes involved in energy storage and expenditure [59-62].

215 In 2004, Backhed et al. [61] reported that conventionally reared mice had a 42% increase in
216 body fat and a 47% increases in periepididymal adipose tissue compared to germ-free mice.
217 Furthermore, transfer of the microbiota from the bowel of the conventional mouse to the gut of the
218 germ-free mouse resulted in a 57% increase in body fat in 2 weeks, although feed consumption
219 decreased. This result highlights the important role that the intestinal microbiota plays in energy
220 homeostasis and its potential involvement in the etiology of obesity. Germ-free mice are resistant to
221 diet -induced adiposity, which is associated with increased activity of AMP-activated protein kinase
222 (AMPK) in liver and muscle and increased expression of adipose factor induced by fasting (Fiaf) in
223 the small intestine [62]. On the other hand, the inoculation of the microbiota of conventional mice fed
224 with this diet into germ-free animals results in an increase in adiposity [59].

225 It should also be noted that the dysbiosis associated with consuming a high-fat diet has been
226 shown to increase intestinal permeability, which results in a greater translocation of LPS from the
227 intestinal lumen to the blood circulation. This metabolic endotoxemia is associated with increased
228 body fat, glucose intolerance and increased expression of proinflammatory mediators and
229 macrophage infiltration in white adipose tissue [60].
230

231 **3. Toll-Like Receptor 4 and Inflammatory Response**
232
233 The innate immune systems of mammals—which encompasses cells such as neutrophils and
234 macrophages—use different strategies to recognize microorganisms. One of these strategies is based
235 on recognizing general aspects of molecules associated with pathogens (*pathogen-associated molecular*
236 *patterns*, or PAMPs) that result from microbial metabolism conserved throughout the evolution of
237 the species. These molecules are widely distributed among pathogens; for instance, the LPS
238 molecule is common in all Gram-negative bacteria, although it is not produced by the host [63-65].
239 Innate immune system receptors capable of recognizing PAMPs are called pattern
240 recognition receptors and these induce the expression of pro-inflammatory cytokines—for example,
241 TNF-α and IL-1β—as well as activating the host's antimicrobial defense mechanisms, such as the
242 synthesis of reactive oxygen and nitrogen species, including hydrogen peroxide and nitric oxide
243 (NO), respectively [66,67]. PAMP recognition can induce CD80 and CD86 costimulatory molecules
244 on the surface of cells, presenting antigens as well as inducing small antigenic peptides linked to
245 MHC class II molecules in cell membranes that present antigens to $CD4^+$ T lymphocytes so activating
246 adaptive immune responses [68].
247 The innate immune system recognizes PAMPs through toll-like receptors (TLRs) that are a
248 family of transmembrane proteins responsible for playing an essential role in the innate immune
249 system [69]. The main function of the TLR protein lies in controlling inflammatory and
250 immunological responses. TLRs can recognize a whole variety of microbial PAMPs. Eleven different
251 TLRs have been identified in humans and thirteen among all mammals [70]. TLRs belong to the IL-1
252 receptor (IL-1R) superfamily, which have a significant homology in their cytoplasmic regions, such
253 as in the Toll/IL-1R (TIR) domain. The TIR domain is needed for the interaction and recruiting of
254 many adaptive molecules involved in the activation of signaling pathways [67].
255 TLRs are expressed in different cell compartments and are recognized by many PAMPs
256 deriving from viruses, pathogenic bacteria, fungi, and protozoa. TLR1, TLR2, TLR4, TLR5, TLR6,
257 and TLR11 are expressed in the cellular membrane, whereas TLR3, TLR7, TLR8 and TLR9 are
258 expressed in intracellular compartments, such as the endosome and the endoplasmic reticulum.
259 Based on the amino acid sequence and on the genomic structure, TLRs can be divided into five
260 subfamilies: TLR2, TLR3, TLR4, TLR5, and TLR9. The subfamily TLR2 comprises TLR1, TLR2, TLR6
261 and TLR10, whereas the subfamily TLR9 encompasses TLR7, TLR8, and TLR9 [71-73].
262 TLR4 was the first TLR reported in humans; it is expressed in innate immune cells, including
263 monocytes, macrophages, and dendritic cells, as well as in other cell types like adipocytes,
264 enterocytes and muscle cells. As indicated above, LPS is the primary agonist for TLR4 [74]. LPS is an
265 integral structural component found in the external membrane of Gram-negative bacteria as well as
266 representing one of the most powerful microbial inflammation indicators. It is a complex glycolipid
267 composed of one hydrophilic polysaccharide and one hydrophobic domain called lipid A [75]. There
268 is some evidence that saturated fatty acids can also bind to TLR4 and activate TLR4-mediated
269 signaling pathways [76,77]. Also, there are other endogens ligands for TLR4 like heat shock protein
270 (Hsp) 60, Hsp 70, type III repeat extra domain A of fibronectin, oligosaccharides of hyaluronic acid,
271 polysaccharide fragments of heparan sulfate, and fibrinogen [78]. In the context of obesity, the
272 increase in the plasma fibrinogen levels, which represents a positive acute phase protein, acts as a

factor involved in the activation of the TLR4 pathway and, consequently, in the amplification of the inflammatory response [79].

The interaction between LPS and TLR4 induces the synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, and IL-12, which, in turn, work as endogenous inflammatory mediators by interacting with receptors found in different target cells. In addition to cytokines, macrophages release a whole variety of biological mediators in response to LPS, including platelet activation factor, prostaglandins, enzymes, and reactive oxygen and nitrogen species, such as superoxide anion and NO. The synthesis of these pro-inflammatory mediators by monocytes and macrophages is designed to inhibit the growth and dissemination of pathogens and to eliminate them either directly or through induction of adaptive immune responses [63,80].

LPS initially binds to the LPS-binding protein (LBP), which is found in the blood or in extracellular spaces. This protein promotes LPS binding to the CD14 molecule, which, in turn, is moored to the lipid bilayer by means of a glycophosphatidylinositol group found in most cells, except for endothelial ones. CD14 can also exist as a soluble protein and, in this case, can lead LPS to the cell surface. The CD14 molecule is not found in transmembrane and intracellular domains; thus, it cannot trigger signal transduction processes on its own. When LPS binds to CD14, LBP dissociates itself and the LPS-CD14 complex physically associates with TLR4. Such a receptor needs an additional molecule, the so-called extracellular accessory protein (MD2), which binds to the TLR4 extracellular complex in order to recognize LPS [71].

Following ligand binding, TLRs dimerize and undergo conformational changes required for the subsequent recruitment of cytosolic TIR domain-containing adaptor molecules, including the cytoplasmic adapter protein MyD88. The association between TLR4 and MyD88 gathers proteins from the IL-1 receptor associated kinase (IRAK) family. Two members (IRAK4 and IRAK1) are phosphorylated in sequence, and this disrupts them from the receptor complex and promotes their association with TNF receptor associated factor 6 (TRAF6). TRAF6 then activates mitogen activated protein kinase (MAPK) proteins. These kinases can activate the AP-1 transcription factor [81].

The transcription factor NF-κB, which is found in a dimeric form in the cytoplasm of non-stimulated cells, is inactive when associated with κB inhibitors (IκB) (**Figure 2**). The family of IκB proteins includes IκBα, IκBβ, IκBε, and Bcl-3, as well as the carboxy-terminal regions of NF-κB1 (p105) and NF-κB2 (p100). The IκB proteins bind to different NF-κB dimers, although they have different affinities and specificities; therefore, besides the different NF-κB dimers found in a specific cell type, there are a large number of combinations of the IκB and the NF-κB dimers [82,83].

Via MAPK, TRAF6 activates the IκB kinase complex (IKK), which is composed of two catalytic subunits (IKKα and IKKβ) and one regulatory subunit (IKKγ) and has the capacity to induce IκB phosphorylation. This phosphorylation results in IκB dissociation from the NF-κB complex and its subsequent polyubiquitination, which, in turn, leads to IkB degradation (mediated by the 26S proteasome) [73,81]. This process allows the NF-κB dimer to translocate into the nucleus and to activate the transcription of many κB-dependent genes, such as the genes of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, COX-2 and inducible nitric oxide synthase (iNOS) (**Figure 2**). NF-κB also stimulates the synthesis of IκB. Accordingly, the newly synthesized IκB binds to NF-κB and suppresses its activity, providing a feedback inhibition mechanism [74,81]. There are five members of the family of NF-κB transcription factors in mammals: NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel, which can dimerize to form

homodimers and heterodimers that, in turn, are associated with specific transcriptional responses to different stimuli. NF-κB1and NF-κB2 do not contain transcriptional activation domains and their homodimers work as repressors. On the other hand, Rel-A, Rel-B and c-Rel drive the transcriptional activation domain and, except for Rel-B, are capable of forming homodimers and heterodimers along with other members of this family of proteins. Consequently, the balance between different NF-κB homodimers and heterodimers regulates the transcriptional activity level. It is worth highlighting that these proteins are expressed in a specific cell and tissue pattern, which leads to an additional level of regulation. NF-κB1 (p50) and RelA, for example, are broadly expressed and, therefore, the p50/RelA heterodimer is the most common NF-κB-binding activity inducer [82,83].

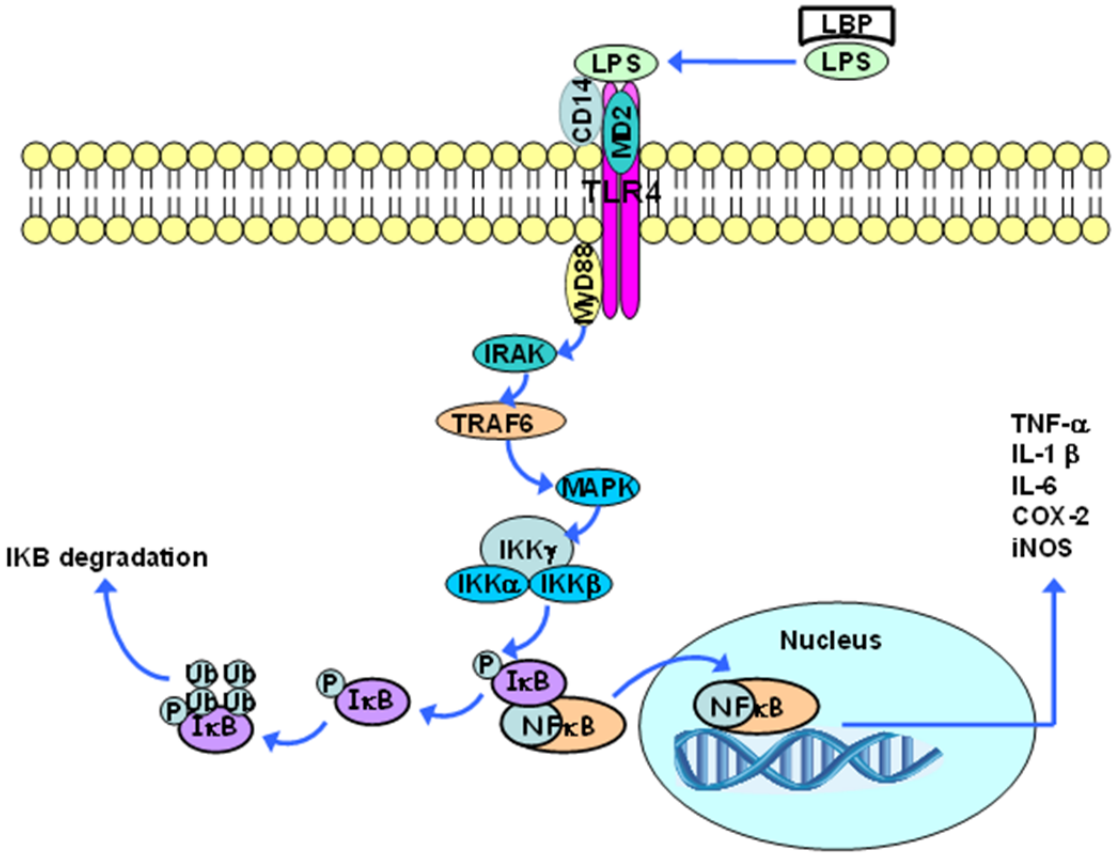


Figure 2. TLR4 induced signaling activates the transcription factor NFκB.

Human monocytes express TLR1, TLR2, TLR4, TLR5, TLR6, TLR8 and TLR9; but TLR2 and TLR4 are the receptors most commonly expressed in these cells. The expression of TLR2 and TLR4 in the plasma membrane of monocytes has been confirmed by flow cytometry; TLR2 and TLR4-binding (by peptidoglycan and LPS, respectively) generates pro-inflammatory cytokine secretion in these cells. Moreover, TLR2 and TLR4 activation recruits monocytes and forms foam cells in murine models of atherosclerosis [30,84].

Studies conducted *in vitro* with cell cultures showed the negative effects of pro-inflammatory cytokines deriving from TLR4 signal pathway activation on glucose uptake and on the metabolism of fatty acids [33,85,86]. TLR4 gene deletion in mice has a protective effect against adipose tissue inflammation and against the resistance to insulin action induced by intake of a high

fat diet, a fact that points towards the causal role played by TLR4 in metabolic changes driven by over-eating and obesity [87,88].

Humans with type I diabetes exhibit a greater expression of TLR2 and TLR4 in the cellular membrane in monocytes, as well as greater MyD88 protein content and IRAK phosphorylation in monocytes in the peripheral blood than in control groups [89]. Individuals with DM2 show increased cellular membrane levels of TLR2 and TLR4 in blood monocytes, as well as a higher concentration of IL-1β, IL-6, IL-8 and TNF-α in serum than in controls [90]. Similarly, TLR2, TLR4 and MyD88 are more highly expressed in blood mononuclear cells and in the abdominal subcutaneous white adipose tissue in obese and diabetic individuals than in patients with normal weight [63,80]. Also, overweight and obese people showed increased expression of TLR2 and TLR4 on peripheral blood mononuclear cells and in adipose tissue in comparison with lean people; the expression levels of TLR2 and TLR4 increased significantly with increasing body mass index [91].

Furthermore, insulin-action resistance in obese individuals can increase the expression of TLR4, which depends on the designated PU.1 transcription factor that, in turn, regulates the gene expression related to the activation and differentiation of myeloid cells, including the TLR2, TLR4, and TLR9 receptors [92,93]. Insulin has a suppressive effect on the expression of TLR4 and on the activity of the PU.1 transcription factor; however, the suppressive effect of the hormone would be expected to be reduced due to the insulin-action resistance related to obesity. Such a reduction would increase the expression of TLR4 in peripheral blood monocytes [94]. In view of this, it seems that the increase of the inflammatory response favors the occurrence of resistance to the action of the insulin, through the activation of the IKK-β and JNK kinases that reduce the activation of IRS-1 in the insulin signaling pathway. Conversely, the presence of insulin resistance favors the expression of TLR4, suggesting that insulin resistance promotes inflammation.

As described earlier, the TLR4 pathway increases the expression of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6, by activating the transcription factors NF-κB and AP-1. These cytokines, in turn, increase the hepatic synthesis of CRP, the classic positive acute phase reactant and the most studied and accepted inflammatory biomarker. CRP is often used in clinical practice due to its high stability (mean half-life of 19 hours) and its rapid production in response to inflammatory stimuli [95,96]. It is important to note that other inflammatory biomarkers, such as IL-6, TNF-α, the intercellular adhesion molecule (ICAM)-1, P-selectin, E-selectin, the monocyte chemotactic protein (MCP)-1, fibrinogen and soluble CD40, have been characterized as predictors of cardiovascular disease, regardless of other cardiovascular risk factors [19,26].

Dietary lipids can cause changes in the expression patterns of TLRs [97]. Ingestion of a high calorie (910 kcal), high lipid (51 g) and high carbohydrate (88 g) meal by normal weight individuals caused significant changes in TLR in the post-prandial period, with TLR2 and TLR4 increasing in blood mononuclear cells. This reinforces the potential importance of postprandial inflammation for obesity, DM2 and cardiovascular disease physiopathology [98,99]. A high-fat meal also leads to increased NF-κB activation in the post-prandial period as well as increased leucocyte activation, as assessed by surface expression of CD11a, CD11b and CD62L [100], and metabolic endotoxemia (i.e. increased plasma LPS levels) [101].

4. Fatty Acids, Toll-Like Receptors and Inflammation

382 *4. 1 Saturated Fatty Acids*

383 Saturated fatty acids, particularly lauric acid and palmitic acid, are capable of stimulating an
384 inflammatory response through the TLR4 signaling pathway [102]. Lee et al. [103] published the first
385 study that demonstrated the effect of different fatty acids on the TLR4 signaling pathway. In this
386 study, it was verified that lauric, palmitic, and stearic acids could induce COX-2 expression through
387 an NFκB-dependent mechanism in a macrophage cell line. Among the saturated fatty acids tested,
388 lauric acid (C12:0) had the greatest activation capacity through TLR4. Different from saturated fatty
389 acids, monounsaturated and polyunsaturated acids did not lead to TLR4 signal activation.
390 Moreover, cell pretreatment *in vitro* for three hours with different polyunsaturated fatty acids,
391 particularly the ω-3 fatty acid docosahexaeanoic acid (DHA: 22: 6 ω-3), or oleic acid (ω-9)
392 significantly reduced the subsequent pro-inflammatory effect induced by lauric acid [103].

393 Saturated fatty acids represent an essential component of bacterial endotoxins. The lipid A
394 portion of LPS has six saturated fatty acids coupled to this structure through ester or amide bonds.
395 The carbon chain length of these fatty acids in lipid A varies from 12 to 16 carbons. Interestingly, the
396 replacement of these saturated fatty acids by monounsaturated or polyunsaturated fatty acids stops
397 the pro-inflammatory activity of the LPS [104].

398 Saturated fatty acids can also induce an inflammatory response through activation of TLR2,
399 which forms heterodimers in the plasma membrane along with TLR1 or TLR6. Diacylated and
400 triacylated lipoproteins, peptidoglycans and lipoteichoic acid are among this receptor's agonists
401 [676,105,106]. Lee et al. [107] reported that lauric acid induced activation through NF-κB when TLR2
402 was cotransfected with TLR1 or TLR6; however, this did not occur when TLR1, 2, 3, 5, 6, or 9 were
403 individually transfected. On the other hand, the omega-3 polyunsaturated fatty DHA suppresses
404 activation through the NF-κB signaling pathway, whether this is induced by LPS or by lauric acid
405 [108]. Furthermore, inhibition of TLR2 expression enhances the sensitivity to insulin action in the
406 skeletal muscle and in the white adipose tissue of mice fed on a high fat diet as well as inhibiting the
407 expression of this receptor. This process results in partial reversal of palmitic acid-induced insulin
408 resistance [23,109].

409 Erridge and Samani [110] suggested that saturated fatty acids would not directly stimulate
410 TRL2 and TLR4 but that this effect could result from the contamination of the bovine serum albumin
411 used to solubilize the saturated fatty acids in the studies conducted *in vitro*. However, Huang et al.
412 [76] demonstrated that saturated fatty acids activate the inflammatory response *in vitro* through
413 TLR2 and TLR4. Lauric acid—which was not solubilized in bovine serum albumin—induced the
414 activation of the NF-κB signaling pathway through TLR2—which was dimerized with TLR1 or
415 TLR6—and TLR4. In addition, there are current propositions addressing TLR4 activation by
416 saturated fatty acids that depend on fetuin A, which is produced in the liver and works through
417 endogenous TLR4-binding [77].

418 Palmitate acid bound to TLR4 activates the kinase proteins JNK and IKK-β and increases the
419 expression and secretion of pro-inflammatory cytokines [86]. Palmitic acid also impairs insulin
420 signaling pathways by inducing IRS-1 phosphorylation at serine residue position 307 [111]. This
421 process reduces its interactions with the insulin receptor and, consequently, diminishes
422 insulin-induced signal transduction. Moreover, saturated fatty acids induce insulin-action resistance
423 due to the antagonistic action of the peroxisome proliferator-activated receptor-gamma coactivator

(PGC)-1 alpha. Such a process induces the expression of mitochondrial genes involved with oxidative phosphorylation and with glucose capture, which is mediated by insulin [112,113].

4.2 Polyunsaturated Fatty Acids

Polyunsaturated fatty acids consist of two families (ω-3 and ω-6) characterized by the double bond locations defined by the first double bond in relation to the methyl terminal group in the fatty acid molecule. α-Linolenic and linoleic acids are examples of polyunsaturated fatty acids belonging to the ω-3 and ω-6 families, respectively. These two fatty acids are not synthesized in humans, and the lack of ω-3 and ω-6 intake causes signaling and symptom deficits, indicating that such nutrients are essential to humans; therefore, they must be consumed through the diet [24,25,114,115]. However, studies have shown that the ratio of ω-6 to ω-3 fatty acids in the diet has implications for health since increased ratios are associated with an increased risk of chronic disease incidence and progression [116,117].

α-Linolenic acid is the precursor of the ω-3 polyunsaturated fatty acids with a longer chain and a high degree of unsaturation, such as eicosapentaenoic acid (EPA: 20: 5 ω-3) and DHA, which are found in seafood, especially fatty fish, and in fish oil supplements. It is important to note that the α-linolenic concentration in the blood, cells, and tissues is significantly lower than that of the EPA and DHA. This suggests that the primary biological function of α-linolenic is as a substrate in EPA and DHA synthesis [118]. However, evidence shows that α-linolenic conversion into EPA and DHA in humans is relatively low: conversion into EPA is estimated to only be around 8%–12% and conversion into DHA is lower than 1% [119,120].

The beneficial effects resulting from an increased intake of ω-3 fatty acids were originally associated with suppression of thrombosis. However, epidemiologic evidence suggests that the intake of ω-3 fatty acids reduces the morbidity and mortality rates due to cardiovascular diseases, as well as reducing systemic blood pressure, triacylglycerol concentrations and the risk of endothelial dysfunction [27,121-126]. The capacity to lower triacylglycerol concentrations, which is related to diminished hepatic VLDL secretion, stands out among the aforementioned possible metabolic effects resulting from the intake of ω-3 fatty acids. This effect is partially dependent on mechanisms related to nuclear receptors, particularly the peroxisome proliferator activated receptor (PPAR)-α [127].

An increased intake of ω-3 fatty acids results in the corresponding accumulation of these fatty acids in cell membranes and circulating lipids. They replace ω-6 fatty acids (such as linoleic and arachidonic acids) in blood lipids and in cell membranes and also modulate/activate different signaling pathways [128].

The ω-3 and ω-6 polyunsaturated fatty acids generate relevant modulations in the inflammatory response because they are precursors to different series of eicosanoids, which have different effects on the intensity of the inflammatory response. Accordingly, ω-6 arachidonic acid generates even-series eicosanoids, such as prostaglandin E_2 and leukotriene B_4. These eicosanoids induce pro-inflammatory effects, such as increased vascular permeability, vasodilation, fever and chemotaxis. It is important to note that prostaglandin E_2 also has anti-inflammatory effects, such as reduced IL-1 and TNF-α production. EPA is the precursor for odd-series eicosanoids, such as prostaglandin E_3, thromboxane A_3 and leukotriene B_5, which induce lower-intensity inflammatory responses. Leukotriene B_5, for example, is 10 to 100 times less potent as a chemotactic agent in neutrophils than leukotriene B_4 [23,27,129]. EPA also competes with arachidonic acid for COX-2 and

5-LOX; therefore, EPA reduces the synthesis of even-series eicosanoids [130]. In addition, higher EPA and DHA concentrations in the plasma membrane favor the production of mediators such as resolvins, maresins and protectins, which are involved in resolution of inflammation and healing [21,25,131].

The ingestion of alpha-linolenic acid can also modulate on the inflammatory response in humans. For example, Caughey et al. [132] observed a significant reduction of TNF-α, IL-1β, TXB_2 and PGE_2 production by LPS-stimulated mononuclear cell cultures obtained from healthy subjects who consumed approximately 14 g/d alpha-linolenic acid for 4 weeks compared to baseline and to a control group. The effect of α-linolenic acid may have been mediated through its conversion to EPA.

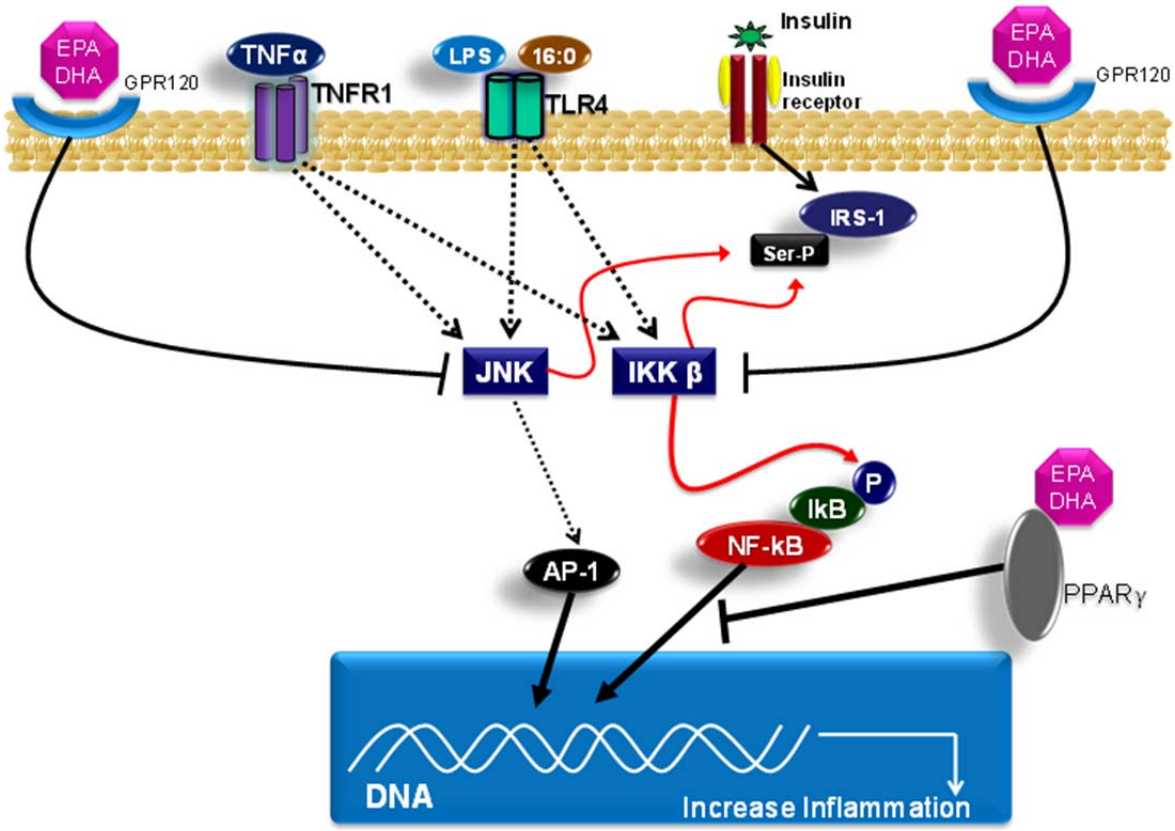
With regard to the molecular effects of EPA and DHA on inflammatory-response modulation, studies have shown that these fatty acids inhibit the expression of inflammatory genes, such as COX-2, iNOS and IL-1 in macrophages [103,108]. In contrast to the stimulating effect of saturated fatty acids on TLR2 and TLR4 activation, EPA and DHA are capable of mitigating the activation of the NF-κB transcription factor pathway induced by various agonists [103,133,134]. Thus, DHA reduces NF-κB pathway activation and the expression of cytokines and COX-2 induced by TLR agonists, such as lipopeptides (TLR2) and LPS (TLR4) in macrophages [89]. In addition, there is reduced gene expression of COX-2 induced by LPS in monocytes from the peripheral blood of individuals who use fish oil supplements [103,108]. The synthesis of the cytokines IL-1, IL-2 and TNF-α was also mitigated after stimulation with LPS *in vitro* by mononuclear cells from the peripheral blood from individuals supplemented with 18 g of fish oil per day for six weeks [135].

In addition, EPA and DHA present another mechanism to modulate the inflammatory response by binding to G-protein coupled receptor 120 (GPR120) which is also known as free fatty acid receptor 4 (FFA4). GPR120 activation induced by EPA or DHA leads to β-arrestin 2 recruitment to the plasma membrane, where this protein binds to GPR120. Subsequently, the GPR120/β-arrestin 2 complex is internalized into the cytoplasmic compartment, where this complex binds to the TAK1-binding protein (TAB1). This process impairs the association between TAB1 and the kinase activated by the growth factor beta (TAK1) and, consequently, results in reduced TAK1 activation and in reduced activity of the IKK-β/NF-κB and JNK/AP-1 signaling pathways. Accordingly, the TAB1/TAK1 binding is a convergence point of stimuli induced by the TLR4 signaling pathway and of the TNF receptor (TNFR). The mitigation of TAK-1 activation by DHA leads to reduced expression of genes with pro-inflammatory actions, such as TNF-α and IL-6 [136,137].

Other mechanisms related to the EPA and DHA effects concern their capacities to bind to peroxisome proliferator activated receptors (PPARs), including the isoforms PPAR-alpha, PPAR-gamma and PPAR-beta/delta. PPARs are a group of nuclear receptors coded for by different genes. PPAR isoforms form heterodimers with the retinoid X receptor (RXR) and bind to peroxisome proliferator response elements (PPRE) in the region responsible for promoting the target genes involved in lipid metabolism and in the inflammatory response; subsequently, they modulate the expression of these genes [138]. PPAR-alpha and PPAR-gamma activations reduce the expression of genes that code for proteins presenting pro-inflammatory actions through inhibition of NF-κB activation. It is worth emphasizing that EPA and DHA directly interact with PPARs and, therefore, modulate the expression of genes involved in lipid metabolism and the inflammatory response [139]. Furthermore, the anti-inflammatory effects of EPA and DHA on this signaling pathway can occur due to diminished nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity,

510 which leads to lower TLR4 recruitment for lipid rafts and TLR4 dimerization [102]. Moreover, the
511 lower NADPH oxidase activity also decreases the production of reactive oxygen species, which, in
512 turn, are necessary to activate the TLR4 signaling pathway. Another possible mechanism of action of
513 the ω-3 fatty acids concerns the capacity of incorporating DHA into the plasma membrane, which
514 can lead to reduced TLR4 translocation for lipid rafts formation. This decreases TLR4 pathway
515 activation and, consequently, decreases NF-κB activation [102,140,141].

516 **Figure 3** shows the main molecular mechanisms related to the effects of saturated and
517 omega-3 fatty acids on the TLR4 pathway.



518

519 **Figure 3.** Molecular mechanism of the effects of saturated (16:0) and omega-3 polyunsaturated fatty acids (EPA,
520 DHA) on the TLR4 and NFkB pathways. The arrows → indicate activation and the arrows ⊢ indicate inhibition.
521 Abbreviations: TNFα, Tumor necrosis factor; TNFR1, Tumor necrosis factor receptor 1; LPS,
522 Lipopolysaccharides; 16:0, palmitic acid; TLR4, Toll-like receptor 4; GPR120, G-protein coupled receptor 120;
523 EPA, eicosapentaenoic acid; DHA, Docosahexaenoic acid; IRS-1, Insulin receptor substrate 1; Ser-P,
524 phosphorylated *serine* residues; PPARγ, Peroxisome proliferator-activated receptor gamma; JNK, c-Jun
525 N-terminal kinases; IKK β, inhibitor of nuclear factor kappa-B kinase subunit beta; IkB, NFKB Inhibitor; P,
526 phosphate; AP-1, Activator protein 1.

527

528 **5. Conclusions**

529

530 The inflammatory process that occurs in obese people differs from the classical
531 inflammatory response in certain respects. This inflammatory process manifests itself systemically
532 and is characterized by a chronic low-intensity reaction. In this context, the TLR4 signaling pathway

has been recognized as one of the main triggers in increasing the obesity-induced inflammatory response. This pathway responds to increased exposure to saturated fatty acids and to LPS. Both of these are relevant in the context of obesity, with saturated fatty acids arising from within the adipose tissue triglyceride stores and the LPS arising from increased intestinal permeability perhaps due to an altered gut microbiota. Adipose tissue driven inflammation increases insulin resistance both locally and systemically, so contributing to the co-morbidities of obesity like DM2. Studies indicate that omega-3 fatty acids, namely EPA and DHA, have an anti-inflammatory effect, which involves attenuating the activation of the TLR4 signaling pathway. This has relevant implications for reducing meta-inflammation and, consequently, resistance to insulin action and risk of DM2 and cardiovascular disease in obese individuals. The omega-3 fatty acids can oppose the action of both classic TLR agonists (e.g. LPS) and saturated fatty acids in this regard.

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References

1. Flegal, K.M.; Carroll, M.D.; Kit, B.K.; Ogden, C.L. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA.* **2012**, *307*, 491-497. doi: 10.1001/jama.2012.39.
2. Kopelman, P.G. Obesity as a medical problem. *Nature.* **2000**, *404*, 635-643.
3. Yach, D.; Stuckler, D.; Brownell, K.D. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat. Med.* **2006**, *12*, 62-66.
4. WHO - World Health Organization. **World Health Organization Obesity and overweight Fact Sheet (2016). http://www.who.int/mediacentre/factsheets/fs311/en/**
5. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* **2014**, *384*, 766-781, doi: 10.1016/S0140-6736(14)60460-8.
6. Alexandratos, N.; Bruinsma, J. World Agriculture Towards 2030/2050: the 2012 revision. Rome: FAO; 2012.
7. United Nations News Centre, 2015. United Nations News Centre. http://www.un.org/sustainabledevelopment/blog/2015/07/un-projects-world-population-to-reach-8-5-billion-by-2030-driven-by-growth-in-developing-countries/ (17, January 2018).
8. Ogden, C.L.; Carroll, M.D.; Curtin, L.R.; Lamb, M.M.; Flegal, K.M. Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA.* **2010**, *303*, 242-249, doi: 10.1001/jama.2009.2012.
9. Mokdad, A.H.; Ford, E.S.; Bowman, B.A.; Dietz, W.H.; Vinicor, F.; Bales, V.S.; Marks, J.S. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* **2003**, *289*, 76-79.

10. Daousi, C.; Casson, I.F.; Gill, G.V.; MacFarlane, I.A.; Wilding, J.P.; Pinkney, J.H. Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. *Postgrad. Med. J.* **2006**, *82*, 280-284.
11. Calle, E.E.; Thun, M.J.; Petrelli, J.M.; Rodriguez, C.; Heath, C.W.Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. *N. Engl. J. Med.* **1999**, *341*, 1097-1105.
12. Prospective Studies Collaboration; Whitlock, G.; Lewington, S.; Sherliker, P.; Clarke, R.; Emberson, J.; Halsey, J.; Qizilbash, N.; Collins, R.; Peto, R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet.* **2009**, *373*, 1083-1096, doi:10.1016/S0140-6736(09)60318-4.
13. Amuna, P.; Zotor, F.B. Epidemiological and nutrition transition in developing countries: impact on human health and development. *Proc. Nutr. Soc.* **2008**, *67*, 82-90, doi: 10.1017/S0029665108006058.
14. Vandevijvere, S.; Chow, C.C.; Hall, K.D.; Umali, E.; Swinburn, B.A. Increased food energy supply as a major driver of the obesity epidemic: a global analysis. *Bull. World Health Organ.* **2015**, *93*, 446-456, doi: 10.2471/BLT.14.150565.
15. Roberts, C.K.; Barnard, R.J. Effects of exercise and diet on chronic disease. *J. Appl. Physiol.* (1985). **2005**, *98*, 3-30.
16. Simopoulos, A.P.; DiNicolantonio, J.J. The importance of a balanced ω-6 to ω-3 ratio in the prevention and management of obesity. *Open Heart.* **2016**, *3*, e000385, eCollection 2016.
17. Galli, C.; Calder, P.C. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. *Ann. Nutr. Metab.* **2009**, *55*, 123-139, doi:10.1159/000228999.
18. Calder, P.C. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie.* **2009**, *91*, 791-795, doi: 10.1016/j.biochi.2009.01.008.
19. Calder, P.C.; Ahluwalia, N.; Albers, R.; Bosco, N.; Bourdet-Sicard, R.; Haller, D.; Holgate, S.T.; Jönsson, L.S.; Latulippe, M.E.; Marcos, A.; et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *Br. J. Nutr.* **2013**, *109*, 1-34, doi: 10.1017/S0007114512005119.
20. Molfino, A.; Amabile, M.I.; Monti, M.; Muscaritoli, M. Omega-3 Polyunsaturated Fatty Acids in Critical Illness: Anti-Inflammatory, Proresolving, or Both? *Oxid. Med. Cell Longev.* **2017**, *2017*, 5987082, doi: 10.1155/2017/5987082.
21. Serhan, C.N.; Chiang, N.; Dalli, J. New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. *Mol. Aspects Med.* **2017**, *pii: S0098-2997(17)30091-2*, doi: 10.1016/j.mam.2017.08.002.
22. Calder, P.C. Omega-3 fatty acids and inflammatory processes. *Nutrients,* **2010**, *2*, 355-374, doi: 10.3390/nu2030355.
23. Calder, P.C.; Ahluwalia, N.; Brouns, F.; Buetler, T.; Clement, K.; Cunningham, K.; Esposito, K.; Jönsson, L.S.; Kolb, H.; Lansink, M.; et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br. J. Nutr.* **2011**, *106*, 5-78, doi:10.1017/S0007114511005460.
24. Calder, P.C. The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Mol. Nutr. Food Res.* **2012**, *56*, 1073-1080, doi: 10.1002/mnfr.201100710.
25. Calder, P.C. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim. Biophys. Acta.* **2015**, *1851*, 469-484. doi: 10.1016/j.bbalip.2014.08.010.

26. Calder, P.C. Fatty acids and inflammation: the cutting edge between food and pharma. *Eur. J. Pharmacol.* **2011**, *668*, 50-58, doi: 10.1016/j.ejphar.2011.05.085.
27. Calder, P.C.; Yaqoob, P. Marine omega-3 fatty acids and coronary heart disease. *Curr. Opin. Cardiol.* **2012**, *27*, 412-419, doi: 10.1097/HCO.0b013e328353febd.
28. Emilsson, V.; Thorleifsson, G.; Zhang, B.; Leonardson, A.S.; Zink, F.; Zhu, J.; Carlson, S.; Helgason, A.; Walters, G.B.; Gunnarsdottir, S.; et al. Genetics of gene expression and its effect on disease. *Nature.* **2008**, *452*, 423-428. doi: 10.1038/nature06758.
29. Hotamisligil, G.S.; Shargill, N.S.; Spiegelman, B.M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. **1993**, *259*, 87-91.
30. Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature.* **2006**, *444*, 860-867.
31. Wellen, K.E.; Hotamisligil, G.S. Inflammation, stress, and diabetes. *J. Clin. Invest.* **2005**, *115*, 1111-1119.
32. Zeyda, M.; Stulnig, T.M. Adipose tissue macrophages. *Immunol. Lett.* **2007**, *112*, 61-67.
33. Lumeng, C.N.; Bodzin, J.L.; Saltiel, A.R. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* **2007**, *117*(1), 175-184.
34. Cancello, R.; Clement, K. Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG*. **2006**, *113*(10), 1141-1147.
35. Cave, M.C.; Hurt, R.T.; Frazier,T.H.; Matheson, P.J.; Garrison, R.N.; McClain, C.J.; McClave, S.A. Obesity, inflammation, and the potential application of pharmaconutrition. *Nutr. Clin. Pract.*, **2008**, 23(1), 16-34.
36. Ferrante, A.W. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J. Intern. Med*., **2007**, *262*, 408-414.
37. Cannon, B.; Nedergaard, J. Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **2004**, *84*(1), 277-359.
38. Nedergaard, J.; Bengtsson, T.; Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab*., **2007**, *293*(2), E444–E452.
39. Cinti, S. The adipose organ. *Prostaglandins Leukot. Essent. Fatty Acids.* **2005**, *73*, 9-15.
40. Cao, L.; Choi, E.Y.; Liu, X.; Martin, A.; Wang, C.; Xu, X.; During, M.J. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab.* **2011**, *14*, 324-38.
41. Lowell, B.B.; Susulic, V.; Hamann, A.; Lawitts, J.A.; Himms-Hagen, J.; Boyer, B.B.; Kozak, L.P.; Flier, J.S. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature*. **1993**, *366*, 740-742.
42. Connolly, E.; Morrisey, R.D.; Carnie, J.A. The effect of interscapular brown adipose tissue removal on body-weight and cold response in the mouse. *Br. J. Nutr.* **1982**, *47*, 653-658.
43. van Marken Lichtenbelt, W.D.; Vanhommerig, J.W.; Smulders, N.M.; Drossaerts, J.M.; Kemerink, G.J.; Bouvy, N.D.; Schrauwen, P.; Teule, G.J. Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med*. **2009**, *360*, 1500-1508.
44. Nisoli, E.; Briscini, L.; Giordano, A.; Tonello, C.; Wiesbrock, S.M.; Uysal, K.T.; Cinti, S.; Carruba, M.O.; Hotamisligil, G.S. Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity. *Proc. Natl. Acad. Sci. USA*. **2000**, *97*, 8033-8038.

45. Samuel, V.T.; Shulman, G.I. Mechanisms for insulin resistance: common threads and missing links. *Cell.* **2012**, *148*, 852-871, doi: 10.1016/j.cell.2012.02.017.
46. Guo, S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. *J. Endocrinol.* **2014**, *220*, 1-23, doi: 10.1530/JOE-13-0327.
47. Hotamisligil, G.S.; Davis, R.J. Cell Signaling and Stress Responses. *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, pii: a006072, doi: 10.1101/cshperspect.a006072.
48. Han, M.S.; Jung, D.Y.; Morel, C.; Lakhani, S.A.; Kim, J.K.; Flavell, R.A.; Davis, R.J. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science.* **2013**, *339*, 218-222, doi: 10.1126/science.1227568.
49. Tilg, H.; Moschen, A.R. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends Endocrinol. Metab.* **2008**, *19*, 371-379, doi: 10.1016/j.tem.2008.08.005.
50. Kirwan, A.M.; Lenighan, Y.M.; O'Reilly, M.E.; McGillicuddy, F.C.; Roche, H.M. Nutritional modulation of metabolic inflammation. *Biochem. Soc. Trans.* **2017**, *45*, 979-985, doi: 10.1042/BST20160465.
51. Egger, G.; Dixon, J. Obesity and chronic disease: always offender or often just accomplice? *Br. J. Nutr.* **2009**, *102*, 1238-42, doi: 10.1017/S0007114509371676.
52. Ertunc, M.E.; Hotamisligil, G.S. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J. Lipid Res.* **2016**, *57*, 2099-2114.
53. Hotamisligil, G.S. Inflammation, metaflammation and immunometabolic disorders. *Nature.* **2017**, *542*, 177-185. doi: 10.1038/nature21363.
54. Cai, D.; Yuan, M.; Frantz, D.F.; Melendez, P.A.; Hansen, L.; Lee, J.; Shoelson, S.E. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat. Med.* **2005**, *11*, 183-190.
55. Ehses, J.A.; Perren, A.; Eppler, E.; Ribaux, P.; Pospisilik, J.A.; Maor-Cahn, R.; Gueripel, X.; Ellingsgaard, H.; Schneider, M.K.; Biollaz, G.; et al. Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes.* **2007**, *56,* 2356-2370.
56. De Souza, C.T.; Araujo, E.P.; Bordin, S.; Ashimine, R.; Zollner, R.L.; Boschero, A.C.; Saad, M.J.; Velloso, L.A. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology.* **2005**, *146*, 4192-4199.
57. Milanski, M.; Arruda, A.P.; Coope, A.; Ignacio-Souza, L.M.; Nunez, C.E.; Roman, E.A.; Romanatto, T.; Pascoal, L.B.; Caricilli, A.M.; Torsoni, M.A.; et al. Inhibition of hypothalamic inflammation reverses diet-induced insulin resistance in the liver. *Diabetes.* **2012**, *61*, 1455-1462, doi: 10.2337/db11-0390.
58. Varma, V.; Yao-Borengasser, A.; Rasouli, N.; Nolen, G.T.; Phanavanh, B.; Starks, T.; Gurley, C.; Simpson, P.; McGehee, R.E. Jr.; Kern, P.A.; et al. Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. *Am. J. Physiol. Endocrinol. Metab.* **2009**, *296*, 1300-1310, doi: 10.1152/ajpendo.90885.2008.
59. Turnbaugh, P.J.; Bäckhed, F.; Fulton, L.; Gordon, J.I. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*. **2008**, *3*(4), 213-223.
60. Cani, P.D.; Bibiloni, R.; Knauf, C.; Neyrinck, A.M.; Delzenne, N.M.; Burcelin, R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes.* **2008**, *57*(6), 1470-1481.

690 61. Backhed, F.; Ding, H.; Wang, T.; Hooper, L.V.; Koh, G.Y.; Nagy, A.; Semenkovich, C.F.; Gordon, J.I. The
691 gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA*. **2004**,
692 *101*(44), 15718-15723.
693 62. Bäckhed, F.; Manchester, J.K.; Semenkovich, C.F.; Gordon, J.I. Mechanisms underlying the resistance to
694 diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. USA*. **2007**, *104*(3), 979-984.
695 63. Basith, S.; Manavalan, B.; Lee, G.; Kim, S.G.; Choi, S. Toll-like receptor modulators: a patent review
696 (2006-2010). *Expert Opin. Ther. Pat.* **2011**, *21*, 927-944, doi:10.1517/13543776.2011.569494.
697 64. Pandey, S.; Kawai, T.; Akira, S. Microbial sensing by Toll-like receptors and intracellular nucleic acid
698 sensors. *Cold Spring Harb. Perspect. Biol.* **2014**, *7*, a016246, doi: 10.1101/cshperspect.a016246.
699 65. Satoh, T.; Akira, S. Toll-Like Receptor Signaling and Its Inducible Proteins. *Microbiol. Spectr.* **2016**, *4*, doi:
700 10.1128/microbiolspec.MCHD-0040-2016.
701 66. Beutler, B.A. TLRs and innate immunity. *Blood.* **2009**, *113*, 1399-1407, doi:10.1182/blood-2008-07-019307.
702 67. Moresco, E.M.; LaVine, D.; Beutler, B. Toll-like receptors. *Curr. Biol.* **2011**, *21*, 488-493. doi:
703 10.1016/j.cub.2011.05.039.
704 68. Lin, Q.; Li, M.; Fang, D.; Fang, J.; Su, S.B. The essential roles of Toll-like receptor signaling pathways in
705 sterile inflammatory diseases. *Int. Immunopharmacol.* **2011**, *11*, 1422-1432, doi: 10.1016/j.intimp.2011.04.026.
706 69. Carvalho, F.A.; Aitken, J.D.; Vijay-Kumar, M.; Gewirtz, A.T. Toll-like receptor-gut microbiota interactions:
707 perturb at your own risk! *Annu. Rev. Physiol.* **2012**, *74*, 177-198. doi:
708 10.1146/annurev-physiol-020911-153330.
709 70. Trudler, D.; Farfara, D.; Frenkel, D. Toll-like receptors expression and signaling in glia cells in
710 neuro-amyloidogenic diseases: towards future therapeutic application. *Mediators Inflamm.* ***2010***, *2010*, pii:
711 497987, doi: 10.1155/2010/497987.
712 71. Connolly, D.J.; O'Neill, L.A. New developments in Toll-like receptor targeted therapeutics. *Curr. Opin.*
713 *Pharmacol.* **2012**, *12*, 510-518. doi: 10.1016/j.coph.2012.06.002.
714 72. Triantafilou, M.; Triantafilou, K. The dynamics of LPS recognition: complex orchestration of multiple
715 receptors. *J. Endotoxin Res.* **2005**, *11*, 5-11.
716 73. Beutler, B. Innate immunity: an overview. *Mol. Immunol.* **2004**, *40*, 845-859.
717 74. Dobrovolskaia, M.A.; Vogel, S.N. Toll receptors, CD14, and macrophage activation and deactivation by
718 LPS. *Microbes Infect.* **2002**, *4*, 903-914.
719 75. Triantafilou, M.; Triantafilou, K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation
720 cluster. *Trends Immunol.* **2002**, *23*, 301-304.
721 76. Huang, S.; Rutkowsky, J.M.; Snodgrass, R.G.; Ono-Moore, K.D.; Schneider, D.A.; Newman, J.W.; Adams,
722 S.H.; Hwang, D.H. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. *J.*
723 *Lipid Res.* **2012**, *53*, 2002-2013, doi:10.1194/jlr.D029546.
724 77. Pal, D.; Dasgupta, S.; Kundu, R.; Maitra, S.; Das, G.; Mukhopadhyay, S.; Ray, S.; Majumdar, S.S.;
725 Bhattacharya, S. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin
726 resistance. *Nat. Med.* **2012**, *18*, 1279-1285, doi: 10.1038/nm.2851.
727 78. Gay, N.J.; Gangloff, M. Structure and function of Toll receptors and their ligands. *Annu. Rev. Biochem.* **2007**,
728 *76*, 141-165.

729 79. Al-ofi, E.; Coffelt, S.B.; Anumba, D.O. Fibrinogen, an endogenous ligand of Toll-like receptor 4, activates
730 monocytes in pre-eclamptic patients. *J. Reprod. Immunol.* **2014,** *103*, 23-28. doi: 10.1016/j.jri.2014.02.004.
731 80. Könner, A.C.; Brüning, J.C. Toll-like receptors: linking inflammation to metabolism. *Trends Endocrinol.*
732 *Metab.* **2011**, *22*, 16-23, doi: 10.1016/j.tem.2010.08.007.
733 81. Kawai, T.; Akira, S. Pathogen recognition with Toll-like receptors. *Curr. Opin. Immunol.* **2005**, *17*, 338-344.
734 82. Caamaño, J.; Hunter, C.A. NF-kappaB family of transcription factors: central regulators of innate and
735 adaptive immune functions. *Clin. Microbiol. Rev.* **2002**, *15*, 414-429.
736 83. Li, Q.; Verma, I.M. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol.* **2002**, *2*, 725-734.
737 84. Cole, J.E.; Georgiou, E.; Monaco, C. The expression and functions of toll-like receptors in atherosclerosis.
738 *Mediators Inflamm.* **2010**, *2010*, 393946, doi:10.1155/2010/393946.
739 85. Jager. J.; Grémeaux, T.; Cormont, M.; Le Marchand-Brustel, Y.; Tanti, J.F. Interleukin-1beta-induced
740 insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression.
741 *Endocrinology.* **2007**, *148*, 241-251.
742 86. Kim, F.; Pham, M.; Luttrell, I.; Bannerman, D.D.; Tupper, J.; Thaler, J.; Hawn, T.R.; Raines, E.W.; Schwartz,
743 M.W. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity.
744 *Circ. Res.* **2007**, *100*, 1589-1596.
745 87. Saberi, M.; Woods, N.B.; de Luca, C.; Schenk, S.; Lu, J.C.; Bandyopadhyay, G.; Verma, I.M.; Olefsky, J.M.
746 Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin
747 resistance in high-fat-fed mice. *Cell Metab.* **2009**, *10*, 419-429, doi: 10.1016/j.cmet.2009.09.006.
748 88. Shi, H.; Kokoeva, M.V.; Inouye, K.; Tzameli, I.; Yin, H.; Flier, J.S. TLR4 links innate immunity and fatty
749 acid-induced insulin resistance. *J. Clin. Invest.* **2006**, *116*, 3015-3025.
750 89. Devaraj, S.; Dasu, M.R.; Rockwood, J.; Winter, W.; Griffen, S.C.; Jialal, I. Increased toll-like receptor (TLR) 2
751 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a
752 proinflammatory state. *J. Clin. Endocrinol. Metab.* **2008**, *93*, 578-583.
753 90. Ahmad, R.; Al-Mass, A.; Atizado, V.; Al-Hubail, A.; Al-Ghimlas, F.; Al-Arouj, M.; Bennakhi, A.; Dermime,
754 S.; Behbehani, K. Elevated expression of the toll like receptors 2 and 4 in obese individuals: its significance
755 for obesity-induced inflammation. *J. Inflamm. (Lond).* **2012,** *9*(1), 48. doi: 10.1186/1476-9255-9-48.
756 91. Dasu, M.R.; Devaraj, S.; Park, S.; Jialal, I. Increased toll-like receptor (TLR) activation and TLR ligands in
757 recently diagnosed type 2 diabetic subjects. *Diabetes Care.* **2010**, *33*, 861-868. doi: 10.2337/dc09-1799.
758 92. Haehnel, V.; Schwarzfischer, L.; Fenton, M.J.; Rehli, M. Transcriptional regulation of the human toll-like
759 receptor 2 gene in monocytes and macrophages. *J. Immunol.* **2002**, *168*, 5629-5637.
760 93. Rehli, M.; Poltorak, A.; Schwarzfischer, L.; Krause, S.W.; Andreesen, R.; Beutler, B. PU.1 and interferon
761 consensus sequence-binding protein regulate the myeloid expression of the human Toll-like receptor 4
762 gene. *J. Biol. Chem.* **2000**, *275*, 9773-9781.
763 94. Ghanim, H.; Mohanty, P.; Deopurkar, R.; Sia, C.L.; Korzeniewski, K.; Abuaysheh, S.; Chaudhuri, A.;
764 Dandona, P. Acute modulation of toll-like receptors by insulin. *Diabetes Care.* **2008**, *31*, 1827-1831, doi:
765 10.2337/dc08-0561.
766 95. Wang, C. Obesity, inflammation, and lung injury (OILI): the good. *Mediators Inflamm.* **2014**, *2014*, 978463,
767 doi: 10.1155/2014/978463.

96. Wu, Y.; Potempa, L.A.; El Kebir, D.; Filep, J.G. C-reactive protein and inflammation: conformational changes affect function. *Biol. Chem.* **2015**, *396*, 1181-1197, doi:10.1515/hsz-2015-0149.
97. Aljada, A.; Mohanty, P.; Ghanim, H.; Abdo, T.; Tripathy, D.; Chaudhuri, A.; Dandona, P. Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. *Am. J. Clin. Nutr.* **2004**, *79*, 682-690.
98. Ghanim, H.; Abuaysheh, S.; Sia, C.L.; Korzeniewski, K.; Chaudhuri, A.; Fernandez-Real, J.M.; Dandona, P. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care.* **2009**, *32*, 2281-2287, doi: 10.2337/dc09-0979.
99. Burdge, G.C.; Calder, P.C. Plasma cytokine response during the postprandial period: a potential causal process in vascular disease? *Br. J. Nutr.* **2005**, *93*, 3-9.
100. van Oostrom, A.J.; Rabelink, T.J.; Verseyden, C.; Sijmonsma, T.P.; Plokker, H.W.; De Jaegere, P.P.; Cabezas, M.C. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis.* **2004**, *177*, 175-182.
101. Erridge, C.; Attina, T.; Spickett, C.M.; Webb, D.J. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.* **2007**, *86*, 1286-1292.
102. Hwang, D.H.; Kim, J.A.; Lee, J.Y. Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *Eur. J. Pharmacol.* **2016**, *785*, 24-35, doi: 10.1016/j.ejphar.2016.04.024.
103. Lee, J.Y.; Sohn, K.H.; Rhee, S.H.; Hwang, D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J. Biol. Chem.* **2001**, *276*, 16683-16689.
104. Hoshino, K.; Takeuchi, O.; Kawai, T.; Sanjo, H.; Ogawa, T.; Takeda, Y.; Takeda, K.; Akira, S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J. Immunol.* **1999**, *162,* 3749-3752.
105. Mylona, E.E.; Mouktaroudi, M.; Crisan, T.O.; Makri, S.; Pistiki, A.; Georgitsi, M.; Savva, A.; Netea, M.G.; van der Meer, J.W.; Giamarellos-Bourboulis, E.J.; et al. Enhanced interleukin-1β production of PBMCs from patients with gout after stimulation with Toll-like receptor-2 ligands and urate crystals. *Arthritis Res. Ther.* **2012**, *14*, 158, doi: 10.1186/ar3898.
106. Snodgrass, R.G.; Huang, S.; Choi, I.W.; Rutledge, J.C.; Hwang, D.H. Inflammasome-mediated secretion of IL-1β in human monocytes through TLR2 activation; modulation by dietary fatty acids. *J. Immunol.* **2013**, *191*, 4337-4347, doi: 10.4049/jimmunol.1300298.
107. Lee, J.Y.; Zhao, L.; Youn, H.S.; Weatherill, A.R.; Tapping, R.; Feng, L.; Lee, W.H.; Fitzgerald, K.A.; Hwang, D.H. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. *J. Biol. Chem.* **2004**, *279*, 16971-16979.
108. Lee, J.Y.; Plakidas, A.; Lee, W.H.; Heikkinen, A.; Chanmugam, P.; Bray, G.; Hwang, D.H. Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. *J. Lipid Res.* **2003**, *44*, 479-486.

109. Caricilli, A.M.; Nascimento, P.H.; Pauli, J.R.; Tsukumo, D.M.; Velloso, L.A.; Carvalheira, J.B.; Saad, M.J. Inhibition of toll-like receptor 2 expression improves insulin sensitivity and signaling in muscle and white adipose tissue of mice fed a high-fat diet. *J. Endocrinol.* **2008**, *199*, 399-406. doi: 10.1677/JOE-08-0354.
110. Erridge, C.; Samani, N.J. Saturated fatty acids do not directly stimulate Toll-like receptor signaling. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 1944-1949, doi: 10.1161/ATVBAHA.109.194050
111. Capurso, C.; Capurso, A. From excess adiposity to insulin resistance: the role of free fatty acids. *Vascul. Pharmacol.* **2012**, *57*, 91-97. doi: 10.1016/j.vph.2012.05.003.
112. Holland, W.L.; Bikman, B.T.; Wang, L.P.; Yuguang, G.; Sargent, K.M.; Bulchand, S.; Knotts, T.A.; Shui, G.; Clegg, D.J.; Wenk, M.R.; et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J. Clin. Invest.* **2011**, *121*, 1858-1870, doi: 10.1172/JCI43378.
113. Patel, P.S.; Buras, E.D.; Balasubramanyam, A. The role of the immune system in obesity and insulin resistance. *J. Obes.* **2013**, *2013*, 616193, doi: 10.1155/2013/616193.
114. Calder, P.C. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci. (Lond).* **2004**, *107*, 1-11.
115. Calder, P.C.; Yaqoob, P. Understanding omega-3 polyunsaturated fatty acids. *Postgrad. Med.* **2009**, *121*, 148-157, doi: 10.3810/pgm.2009.11.2083.
116. Burghardt, P.R.; Kemmerer, E.S.; Buck, B.J.; Osetek, A.J.; Yan, C.; Koch, L.G.; Britton, S.L.; Evans, S.J. Dietary n-3:n-6 fatty acid ratios differentially influence hormonal signature in a rodent model of metabolic syndrome relative to healthy controls. *Nutr. Metab. (Lond).* **2010**, *7*, 53, doi: 10.1186/1743-7075-7-53.
117. Wan, J.B.; Huang, L.L.; Rong, R.; Tan, R.; Wang, J.; Kang, J.X. Endogenously decreasing tissue n-6/n-3 fatty acid ratio reduces atherosclerotic lesions in apolipoprotein E-deficient mice by inhibiting systemic and vascular inflammation. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 2487-2494, doi: 10.1161/ATVBAHA.110.210054.
118. Baker, E.J.; Miles, E.A.; Burdge, G.C.; Yaqoob, P.; Calder, P.C. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog. Lipid Res.* **2016**, *64*, 30-56, doi: 10.1016/j.plipres.2016.07.002.
119. Burdge, G.C.; Wootton, S.A. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.* **2002**, *88*, 411-420.
120. Goyens, P.L.; Spilker, M.E.; Zock, P.L.; Katan, M.B.; Mensink, R.P. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am. J. Clin. Nutr.* **2006**, *84*, 44-53.
121. Calder, P.C. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins Leukot. Essent. Fatty Acids.* **2008**, *79*, 101-108, doi: 10.1016/j.plefa.2008.09.016.
122. Casula, M.; Soranna, D.; Catapano, A.L.; Corrao, G. Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, placebo controlled trials [corrected]. *Atheroscler. Suppl.* **2013**, *14*, 243-251. doi: 10.1016/S1567-5688(13)70005-9.
123. Chowdhury, R.; Warnakula, S.; Kunutsor, S.; Crowe, F.; Ward, H.A.; Johnson, L.; Franco, O.H.; Butterworth, A.S.; Forouhi, N.G.; Thompson, S.G.; et al. Association of dietary, circulating, and supplement

fatty acids with coronary risk: a systematic review and meta-analysis. *Ann. Intern. Med.* **2014**, *160*, 398-406. doi: 10.7326/M13-1788.

124. Marik, P.E.; Varon, J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin. Cardiol.* **2009**, *32*, 365-372, doi: 10.1002/clc.20604.
125. Studer, M.; Briel, M.; Leimenstoll, B.; Glass, T.R.; Bucher, H.C. Effect of different antilipidemic agents and diets on mortality: a systematic review. *Arch. Intern. Med.* **2005**, *165*, 725-730.
126. Yokoyama, M.; Origasa, H.; Matsuzaki, M.; Matsuzawa, Y.; Saito, Y.; Ishikawa, Y.; Oikawa, S.; Sasaki, J.; Hishida, H.; Itakura, H.; et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet.* **2007**, *369*, 1090-1098.
127. Buettner, R.; Parhofer, K.G.; Woenckhaus, M.; Wrede, C.E.; Kunz-Schughart, L.A.; Schölmerich, J.; Bollheimer, L.C. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.* **2006**, *36*, 485-501.
128. Hartweg, J.; Farmer, A.J.; Holman, R.R.; Neil, A. Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes. *Curr. Opin. Lipidol.* **2009**, *20*, 30-38.
129. Yu, K.; Bayona, W.; Kallen, C.B.; Harding, H.P.; Ravera, C.P.; McMahon, G.; Brown, M.; Lazar, M.A. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* **1995**, *270*, 23975-23983.
130. Kalupahana, N.S.; Claycombe, K.; Newman, S.J.; Stewart, T.; Siriwardhana, N.; Matthan, N.; Lichtenstein, A.H.; Moustaid-Moussa, N. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J. Nutr.* **2010**, *140*, 1915-1922, doi:10.3945/jn.110.125732.
131. Caughey, G.E.; Mantzioris, E.; Gibson, R.A.; Cleland, L.G.; James, M.J. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *Am. J. Clin. Nutr.* **1996**, *63*, 116-122.
132. Chiang, N.; Serhan, C.N. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. *Mol. Aspects Med.* **2017**, *58*, 114-129, doi: 10.1016/j.mam.2017.03.005.
133. Sampath, H.; Ntambi, J.M. Polyunsaturated fatty acid regulation of gene expression. *Nutr. Rev.* **2004**, *62*, 333-339.
134. Stryjecki, C.; Mutch, D.M. Fatty acid-gene interactions, adipokines and obesity. *Eur. J. Clin. Nutr.* **2011**, *65*, 285-297, doi: 10.1038/ejcn.2010.277.
135. Endres, S.; Meydani, S.N.; Ghorbani, R.; Schindler, R.; Dinarello, C.A. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J. Leukoc. Biol.* **1993**, *54*, 599-603.
136. Oh, D.Y.; Talukdar, S.; Bae, E.J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W.J.; Watkins, S.M.; Olefsky, J.M. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* **2010**, *142*, 687-698, doi: 10.1016/j.cell.2010.07.041.
137. Oh, D.Y.; Olefsky, J.M. Omega 3 fatty acids and GPR120. *Cell Metab.* **2012**, *15*, 564-565. doi: 10.1016/j.cmet.2012.04.009.

885 138. Li, A.C.; Glass, C.K. PPAR- and LXR-dependent pathways controlling lipid metabolism and the
886 development of atherosclerosis. *J. Lipid Res.* **2004**, *45*, 2161-2173.
887 139. Martínez-Fernández, L.; Laiglesia, L.M.; Huerta, A.E.; Martínez, J.A.; Moreno-Aliaga, M.J. Omega-3 fatty
888 acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins Other Lipid Mediat.*
889 **2015**, *121*, 24-41, doi: 10.1016/j.prostaglandins.2015.07.003.
890 140. Meital, L.T.; Sandow, S.L.; Calder, P.C.; Russell, F.D. Abdominal aortic aneurysm and omega-3
891 polyunsaturated fatty acids: Mechanisms, animal models, and potential treatment. *Prostaglandins Leukot.*
892 *Essent. Fatty Acids.* **2017**, *118*, 1-9, doi: 10.1016/j.plefa.2017.02.001.
893 141. Puglisi, M.J.; Hasty, A.H.; Saraswathi, V. The role of adipose tissue in mediating the beneficial effects of
894 dietary fish oil. *J. Nutr. Biochem.* **2011**, *22*, 101-108, doi: 10.1016/j.jnutbio.2010.07.003.
895