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Fatty-acid-mediated hypothalamic inflammation and epigenetic programming

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Abstract

A high-fat diet is the main environmental cue that has been studied in the hypothalamus since the discovery of its connection with hypothalamic inflammation. Current evidence shows hypothalamic inflammation as a likely mechanism for the dysregulation on the homeostatic control of energy balance, which leads to metabolic alterations and obesity. Although this mechanism seems to be reversible when set during adulthood, we argue whether dietary fatty acids, during critical periods of development, could affect hypothalamic function permanently and set an increased susceptibility to obesity. We found few experimental studies that looked at programming induced by different fatty acids on the hypothalamus. They clearly showed a connection between maternal fat diet, hypothalamic inflammation and metabolic alterations in the offspring. We found that not only a high-fat diet but also a normolipidic diet with unbalanced quantities of different fatty acids produced diverse inflammatory responses on the hypothalamus. Therefore, strategies of manipulating dietary fatty acids in pregnant and lactating women may have great impact on the population's future health. However, more research is still needed on the effects of fatty acids and the hypothalamic inflammation on programming.

Keywords: Inflammation; Programming; Fatty acids; Obesity; Hypothalamus; Appetite regulation

1. Introduction

The obesity epidemic is a worldwide serious issue and is associated with leading causes of death and disabilities worldwide. Its comorbidities include type 2 diabetes, cardiovascular diseases, musculoskeletal disorders and some cancers. In 2014, statistics revealed that around 600 million people were obese in the world. In addition, children obesity has increased considerably, posing concerns on population's future health, as it is associated with psychological disorders, risk of adulthood obesity and premature deaths. It represents an economic burden for the health care system, having also negative consequences on the country's productivity and welfare [1].

Common sense on how to lose weight tells us that a negative energy balance, eat less and exercise more, does the trick. Unfortunately, this strategy has proved to be ineffective to treat obesity in the long term. Our drive to eat goes beyond willpower, and it is finely regulated by our brain, in particular, the hypothalamus. Neuroscientists are making an effort to unearth the mechanisms that corrupt the homeostatic control of satiety and appetite that leads to weight gain.

Recently, a close correlation has been found between excess of dietary fats, hypothalamic inflammation and the disruption on hypothalamic neural circuits and metabolic alterations [2,3]. This is particularly relevant when we consider that the modern diet shifted to a higher consumption of processed foods, which are rich in fat and sugar and extremely palatable.

However, some people are more prone to obesity than others, and this could be explained by not just their genes themselves but their epigenetic profile. There is evidence that maternal dietary fats can program the offspring, during critical periods of development, and modify its susceptibility to adult chronic diseases [4–7].

The objective of this review is to explore the relationship of fatty acids on hypothalamic inflammation and programming. Our hypothesis is that fatty acids could lead to hypothalamic inflammation, and it is involved in the disruption of normal appetite control, which can lead to obesity. This disruption at an early stage of development could set the susceptibility of the individual to develop noncommunicable diseases in adulthood.

2. Hypothalamic control of energy balance

The hypothalamus is a region in the brain involved in the homeostatic control of food intake and energy expenditure in response to the body's energy state, which is indispensable for survival. The mediobasal hypothalamus (MBH) is the critical area that controls energy balance. In particular, the arcuate nucleus (ARC) contains orexigenic and anorexigenic neurons counterbalancing one another to adjust energy balance. Orexigenic neurons express neuropeptide Y (NPY) and agouti- related peptide (AgRP). Together, these neurotransmitters stimulate food intake, decrease energy expenditure and prevent the anorectic action of the α-melanocyte-stimulating hormone (α-MSH). In contrast, the anorexigenic neuron

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proopiomelanocortin (POMC) expresses the cocaine- and amphetamine-regulated transcript (CART) and POMC, which is cleaved to produce α-MSH that triggers satiety and increases energy expenditure. These hypothalamic neural pathways are interconnected and have projections to other brain areas that influence motivation/reward, energy expenditure, hunger and eating behavior [8].

Neurons in the ARC are sensible to metabolic signals from peripheral tissue, such as leptin and insulin. Leptin is an essential endocrine hormone released by adipocytes, and it mediates the communication between adipose tissue and central nervous system (CNS). Leptin acts on a negative feedback regulation: adipocyte hypertrophy stimulates leptin production. Leptin binds to its receptor in the ARC, suppressing NPY/AgRP and stimulating POMC neurons, causing the end of food intake and increased energy expenditure (Fig. 1). This leads to fat oxidation and reduction in leptin production [9]. Insulin has a similar anorexigenic effect on ARC neurons as leptin [10]. However, both hormones are overexpressed in obese humans, and they display central and peripheral tissue resistance and are linked to the development of metabolic disturbances seen in metabolic diseases.

Low-grade hypothalamic inflammation induced by high-fat diet has recently been under investigation as a possible trigger of hypothalamic insulin and leptin resistance, disrupting the homeostatic regulation of hunger and satiety and altering metabolic control [3,11,12].

2.1. The association between fatty acids and hypothalamic inflammation

In 2005, De Souza et al. [11] were the first to identify hypothalamic inflammation associated with insulin resistance in rats after 16 weeks of a high-fat diet (39% of kcal from lard). Further experiments confirmed these findings and discovered that, unlike peripheral inflammation that occurs after adipocyte hypertrophy, central inflammation induced by a high-fat diet occurs only after 24 h and before weight gain is significant [13]. Therefore, the effects of a high-fat diet seem to initiate in the CNS and affect other tissues only after prolonged consumption of a high-fat diet. Thaler et al. (2012) [13] fed adult rats with a high-fat diet (54% of kcal from lard) for 20 weeks, and they saw increased food intake and weight gain in the treatment group compared to controls. Concomitantly, they found in the treated group an increased expression of proinflammatory markers in the hypothalamus after only 4 weeks on a high-fat diet, which was not seen in the adipose tissue and liver of the animals. Interestingly, these researchers also found that increases in the expression of interleukin (IL)-6, tumor necrosis factor (TNF)-α, suppressor of cytokine signaling 3 (Socs3), I-kappa-B-kinase beta and I-kappa-B kinase epsilon caused a proportionate increase in food intake during the first days. This shows a possible link between hypothalamic inflammation and enhanced energy intake even before the onset of obesity or increased accumulation of adipose tissue.

The mechanisms involved in the hypothalamic inflammation are not completely known, but recent evidence points to gliosis and direct neural injury caused by high-fat diet (high in saturated fats) [2,3,13]. Gliosis is a response of the CNS to neural injury, and it is characterized by recruitment, activation and proliferation of neural-immune cells [14]. Thaler et al. (2012) [13] found increased accumulation, activation and cell size of microglia in the ARC from mice and rats fed high-fat diet, and it positively correlated with fat mass size. Likewise, the same pattern was observed in humans; a retrospective cohort of 34 people free from abnormalities was subjected to magnetic resonance imaging. This identified the presence of gliosis in the MBH, which correlated with BMI. The molecular mechanisms participating in the activation of hypothalamic inflammation are the activation of Toll-like receptor 4 (TLR-4), induction of ER stress and activation of IKKβ [15].

Like macrophages in the periphery, microglia abundantly express TLR-4, a signal-transducing receptor that responds to saturated fats through IKKβ/NFκB pathway to release proinflammatory cytokines (such as IL-6 and TNF-α) [16,17]. TLR-4 is overexpressed in obesity, and its inhibition by intracerebroventricular (ICV) injections, with immunoneutralizing antibodies against TLR-4, brings about the

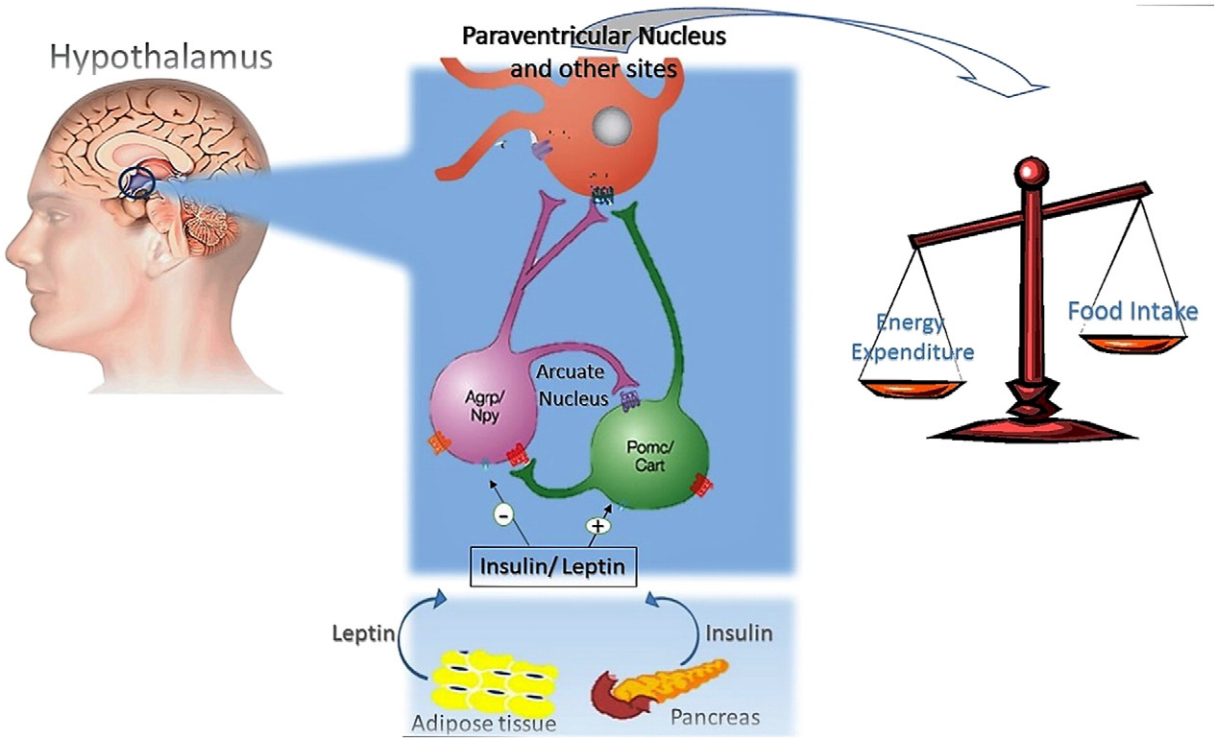

Fig. 1. Control of energy balance by hypothalamic leptin and insulin pathway. Leptin and insulin are released by the adipose tissue and pancreas respectively, on the hypothalamus; they inhibit AGRP/NPY and stimulate POMC/CART neurons. POMC/CART neurons send their projections to paraventricular nucleus and other sites and stimulate cessation of food intake and increase of energy expenditure.

recovery in leptin and insulin signaling and improvement in liver metabolism [18]. Moreover, dietary fats can easily flux into the hypothalamus, and microglia have access to these fats rapidly. Experiments on mice fed saturated fats from milk fat (C16:0 palmitic and C18:0 stearic fatty acids) resulted in increased accumulation of saturated fats in the hypothalamus, accumulation and activation of microglia in the MBH and increased TNF-α release (Fig. 2) [2]. In addition, high-fat diet and saturated fats up-regulate the expression of heat-shock protein 72, a protein involved in the neuronal stress response [2,13]. However, it is still unclear whether the neuronal stress is a result of microglia response to saturated fats or a direct response of neurons to these fatty acids or a combination of both.

A high-fat diet (39% of kcal from lard) and TNF-α can activate neuronal IKKβ/NF-κB pathway, possibly through receptor-independent intracellular organelle stresses and cytokine receptors, respectively. It has been demonstrated that the activation of IKKβ/NF-κB can cause leptin and insulin resistance in the CNS *via* the expression of the SOCS3, a known inhibitor of insulin and leptin signaling (Fig. 2). Suppression of IKKβ on the AGRP neurons interrupts the activation of IKKβ/NF-κB pathway and improves leptin and insulin signaling, protecting against obesity [3].

Besides the mentioned proinflammatory effects of saturated fats on the hypothalamus, other dietary fatty acids can foster distinct inflammatory responses. Partially swapping lard with flax seed oil (rich in polyunsaturated fatty acid C18:3) or olive oil (rich in monounsaturated fatty acid C18:1) reversed diet-induced obesity (DIO) in mice, showing a reversal of hypothalamic inflammation, systemic insulin resistance and body adiposity. Also, ICV injections of ω3 and ω9 fatty acids modify feeding behavior and reduce food intake and adipose tissue accumulation. Additionally, these results showed improvement in insulin/leptin signaling and enhanced anorexigenic and prothermogenic neuropeptides POMC/CART expression while reducing the expression of anabolic neuropeptides NPY and melanin-concentrating hormone [19]. This study showed similar effects between linolenic (ω3) and oleic (ω9) fatty acids. The authors suggest that these fatty acids act upon the unsaturated fatty acid receptor GPR120, which shows increased expression in the NPY neurons [19]. G-protein coupled receptor 120 is activated by unsaturated fats, initially found in monocytes, and represses tissue macrophage inflammation, owing to its insulin-sensitizing and anti-inflammatory actions through β-arrestin 2/TAB1, blocking the TLR4 and TNF inflammatory pathways [20]. Cintra et al. found that despite similar outcomes, ω9 and ω3 fatty acids had some differences in reducing the mediators of inflammation. For instance, ω9 was more potent in reducing iNOS and IL-6, and ω3 fatty acids were more powerful in reducing pJNK. Both reduced TNF-α to a similar degree, and they both showed increased anti-inflammatory cytokine IL-10 [19]. This study shows that a high-fat diet with partial substitution of lard for olive oil or flax seed oil can reverse the hypothalamic inflammation and metabolic alterations seen in DIO.

Similarly, docosahexaenoic acid (DHA) (C22:6n-3) exhibits a potent anti-inflammatory effect on the brain, able to reduce the production of TNF-α and IL-6 by activated microglia [21]. Another study confirmed the protective effect of a high-fish-oil diet (rich in DHA) on hypothalamic metabolic inflammation [22]. However, a high-soy-oil diet (rich in ω 6 PUFA) showed a proinflammatory effect on the hypothalamus of Wistar rats and increased body weight similar to the effects of a high-saturated-fat diet [22]. Likewise, cohort studies analyzing trans-fatty acid intake have linked it to systemic inflammation, endothelial dysfunction and increased risk of stroke [23,24]. In addition, a diet rich in trans-fats during pregnancy and lactation can induce a hypothalamic proinflammatory state and impair central insulin signaling in the offspring [4,5].

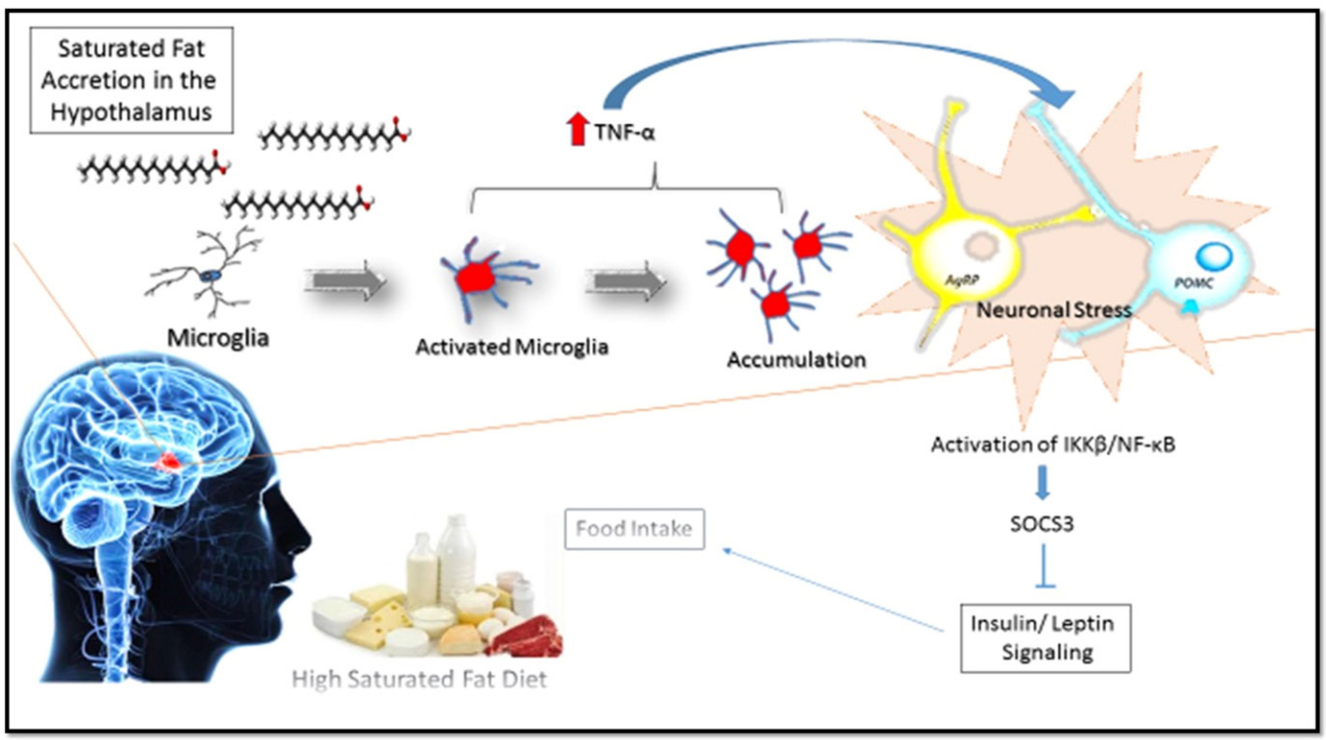

Fig. 2. Mechanism of hypothalamic inflammation induced by saturated fats leading to the disruption of appetite control. High-saturated-fat diet promotes saturated fatty acids accretion in the hypothalamus, resulting in activation and accumulation of microglia and increased TNF-α production, leading to neuronal stress. The activation of IKKβ/NFκB leads to the expression of SOCS3, which inhibits leptin and insulin signaling, leading to increased food intake.

These recent pieces of evidence establish a likely cause and effect relationship between hypothalamic inflammation and the development of metabolic diseases such as obesity. It seems that environmental factors, such as dietetic cues, can trigger central inflammation and alter the body's metabolic homeostatic system.

2.2. Hypothalamic inflammation by fatty acids on programming

Epidemiological studies in the 1900s in Britain associated low birth weights and intrauterine undernutrition with the development of noncommunicable diseases such as type 2 diabetes and cardiovascular disease [25–27]. These observations raised the idea that adverse intrauterine and early postnatal environments have a significant role in shaping or "programming" the developing organs, in critical periods of development, and permanently set an altered physiological response. This leads to dysfunction and determines the susceptibility to adult-onset diseases [28].

A plethora of experimental research in animals supports the hypothesis of the developmental programming of the adult disease [29–31]. Maternal nutrition is one of the most studied environmental factors involved in programming, although the precise molecular mechanisms remain unknown [32].

The mechanism on how the environment modifies the body's phenotype acutely or permanently in short spaces of time can be explained by perturbations during organogenesis which alter the organ structure and function [33] or by epigenetic modifications [34,35]. Epigenetics involve the modification of gene expression while maintaining the same DNA sequence. Epigenetic patterns established during early development can perpetuate for a lifetime and be transmitted to other generations [36]. Main epigenetic marks include changes in methylation of cystine–guanosine (CpG) islands at the promoter region of a target gene and changes in chromatin structure through histone methylation and acetylation [37].

Methylation at the CpG region in the promoter of a specific gene is associated with translational silencing. This can regulate gene expression and eventually influence the phenotype. DNA methylation occurs thanks to the transfer from a methyl donor, catalyzed by DNA methyltransferases (DNMTs), to the CpG region of a gene. Members of DNMT family have different actions; for example, DNMT1 is responsible for the maintenance of methylation patterns during cell replication. DNMT3A, DNMT3B and DNMT3L were found to be involved in *de novo* methylation patterns during germ cell development and embryogenesis [38]. Methylation at the promoter region of a gene can cause silencing by blocking binding sites of transcription factors and also by recruitment of DNA binding proteins such as methyl CpG binding protein 2 and chromatin modification enzymes like histone deacetylases [39].

Moreover, covalent modifications to histone proteins have direct effects on transcriptional activity because of their role in chromatin folding. For instance, histone acetylation at a specific lysine residue unfolds chromatin package, allowing transcriptional factors to bind to the corresponding gene and initiate gene transcriptional activity. In contrast, histone deacetylation causes chromatin folding and condensation, blocking transcriptional activity. Also, the outcome of histone methylation depends on the residue modified; methylation at lysine H3K4 (histone H3 lysine 4) is associated with increased gene expression, while methylation at lysine H3K9 (histone H3 lysine 9) and H3K27 (histone H3 lysine 27) is associated with gene inactivation. Knowledge on the dynamics of histones' covalent modifications is growing, and several enzymes participate in this process. As with DNA methylation, histone modifications are considered potentially inheritable epigenetic marks able to affect biological processes and phenotype [40].

Recent studies searched for epigenetic modifications influenced by an obesogenic environment in early life. They found out that parents' obesity preconceptionally alter methylation signature in newborns, highlighting also the importance of paternal lifestyle and the influence it can possibly exert during gametogenesis [41]. Maternal obesity or gestational diabetes also showed altered placental methylation patterns of leptin, which reflected in its reduced gene expression [42,43]. Maternal obesity is characterized by increased circulating levels of fatty acids, and its supply is mediated by the placenta to assist fetal growth and development [44]. Similar to hypothalamic inflammation in DIO, placenta is also subjected to lipotoxic environment from the obese mother, which is also associated with fetal immune cell recruitment [45], increased inflammatory signals [43], leptin resistance [46] and profound effects on angiogenesis and oxidative stress [43].

Imprinting influenced by early nutrition has also been found in the CNS [47]. Neuronal DNA methylation patterns of dopamine and opioid-related genes, associated with reward and preference for palatable foods, can be programmed by a high-fat (60% of kcal from fat) maternal diet during gestation and lactation in mice. These methylation marks showed long-term behavioral and gene expression alterations in the offspring [47]. Likewise, neonatal overfeeding by breeding Wistar rats in small litters caused hypermethylation of POMC, which led to rapid weight gain and metabolic alterations [31].

There are only a few studies on hypothalamic programming set by fatty acids, and they show a strong connection with a local inflammatory process (Fig. 3). Trans-fatty acids intake during gestation and lactation in Wistar rats leads to offspring's hypothalamic inflammation and wreaks havoc on satiety control even after weaning on standard chow [4]. Another study on the effects of trans-fats in early development showed dampened insulin receptor and insulin substrate 1 levels in the offspring. Interestingly, offspring with a mismatch between intrauterine (dams fed trans-fats) and postweaning environments (control diet) showed lower insulin efficiency and increased glycemia compared to offspring fed a trans-fat diet from their mothers and after weaning. However, the latter showed an alteration in the appetite control, and this could possibly lead to obesity later in adulthood [5]. A comparison between dams' normocaloric diets rich in trans-fat (trans-fat group), palm oil (palm oil group) and interesterified fat (interesterified fat group) showed evidence of immune response and increased TLR4 expression in the offspring's brain in all groups. In adulthood, the animals on interesterified fat group and palm oil group had higher body weights. This raised concerns because palm oil and interesterified fats are largely used as a replacement for trans-fats in processed foods [6]. In contrast, programming study during lactation by low omega 6 or high omega 3 content in the dam's diet caused significant reductions in the density of both orexigenic and anorexigenic neural projections to paraventricular nucleus in the offspring. These alterations persisted during adulthood, and these animals exhibited reduced fat accretion even after being challenged with a Western diet [7].

It is a plausible idea that fatty acids could set hypothalamic inflammation and programming, determining the susceptibility for adult metabolic diseases. The main players in inflammation are the immune cells, and their role in programming still needs to be determined. The placenta takes in maternal circulating fatty acids, but its excess is lipotoxic, and immature fetal macrophage response leads to a proinflammatory state [45]. Inflammation has been associated with programming [4,6]. Prenatal inflammation has also been associated with long-term impaired adult neurogenesis [48]. We speculate whether, at the origins of hypothalamic programming, there is a strong influence of the immune system during early development, which is still immature and may respond to an excess of fatty acids or to different types of fatty acids that accumulate in the tissue, producing proinflammatory cytokines and leading to programming. These early immune cells could also develop a "memory," so called "trained innate immunity," and be less tolerable to certain fatty acids, maintaining a proinflammatory phenotype even during adulthood. This cannot be stated with certainty in this article, but evidence from studies on the arteriosclerosis demonstrate that macrophages *via*

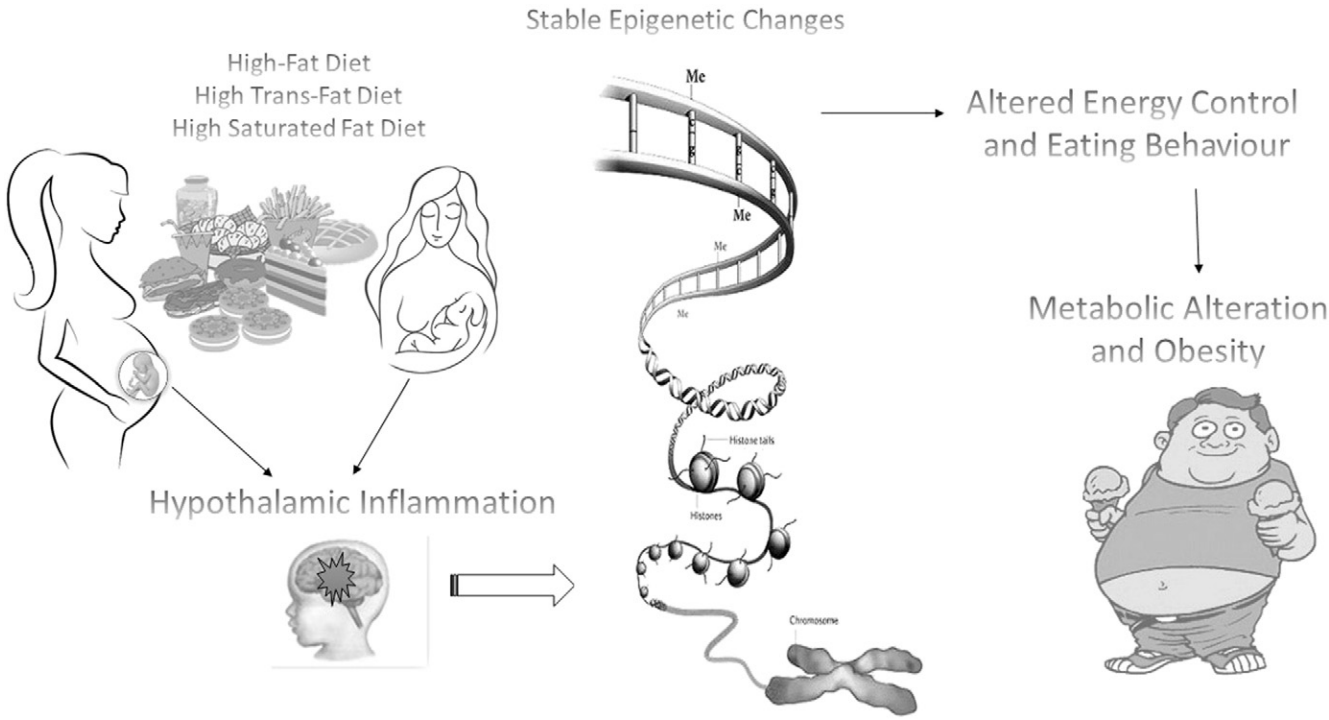

Fig. 3. Mechanism of programming induced by dietary fats. High-fat-diet or high-trans-fat diet or high-saturated-fat diet can cause hypothalamic inflammation, stable epigenetic changes, and altered energy control and eating behavior, consequently leading to metabolic alteration and obesity.

scavenger receptor and TLRs respond to oxidized low-density lipoprotein (oxLDL) producing proinflammatory stimuli [49]. Moreover, oxLDL has shown to cause stable histone modifications in the macrophages, which lead to a long-term proinflammatory phenotype and the production of proaterogenic cytokines and chemokines [50]. Studies on mice showed that bone marrow transplant from hypercholesterolemic to normocholesterolemic mice caused monocytosis and proinflammatory monocytes in the recipient. It suggests that macrophages' "memory" was transmitted to hematopoietic stem cells in the recipient and changed its phenotype to develop more myeloid cells with a strongly marked and persistent proinflammatory response without the causing factor [51,52].

3. Conclusion

Compelling evidence on hypothalamic inflammation induced by saturated fatty acids or high-fat diet shows clearly that hypothalamic inflammation orchestrates metabolic alterations that may initiate obesity. Another key fact to remember is that maternal dietary fatty acids have also been found to induce hypothalamic inflammation, cause epigenetic changes and alter the mechanisms of energy control in the offspring, with lasting effects. It is important to note that not only a high-fat diet but also normolipidic diet with unbalanced quantities of different fatty acids produced inflammatory responses on the hypothalamus. With this in mind, strategies of manipulating dietary fatty acids in pregnant and lactating women may have great impact on the population's future health. Finally, there is a need for further research on the effects of fatty acids on hypothalamic inflammation and epigenetic alterations in early life and observations of the long-term outcomes in adulthood.

Conflicts of interest

None.

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