



Food intake in early life and epigenetic modifications of pro-opiomelanocortin expression in arcuate nucleus

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Abstract

The prevalence of obesity is increasing in nowadays societies and, despite being a multifactorial disease, it has a significant correlation with food intake. The control of food intake is performed by neurons of the arcuate nucleus of the hypothalamus (ARC), which secrete orexigenic and anorexigenic neuropeptides, such as proopiomelanocortin (POMC), under stimulation of, e.g., ghrelin, insulin, and leptin. Insulin, uses inositol 1,4,5-trisphosphate/serine-threonine kinase (IP3/Akt) pathways and stimulates the exclusion of (Forkhead box protein O1) FOXO1 from the nucleus and thereby does the inactivation of the inhibition of POMC expression, while Leptin stimulates signal transducer and activator of transcription 3 (STAT3) phosphorylation and POMC expression. Epigenetic modifications of the synthesis of these neuropeptides can lead to an increased caloric intake, which, in turn, is an important risk factor for obesity and its comorbidities. Epigenetic modifications are reversible, so the search for epigenetic targets has significant scientific and therapeutic appeal. In this review, we synthesize the effect of food intake on the epigenetic modifications of Neuropeptide Y and Pro-opiomelanocortin of ARC and its relationships with obesity development and comorbidities. We found that there is no consensus on the methylation of neuropeptides when the evaluations are carried out in different promoters. Based on reports carried on in the early life in laboratory animals, which is the timeline that the vast majority of author used to study this topic, chronic inflammation, defects in insulin and leptin signaling may be linked to changes occurring in the phosphoinositide 3-kinase/Akt (PI3K/Akt) and/or STAT3/SOCS3 (cytokine signaling 3) pathways. In its turn, the epigenetic modifications related to increased food intake and reduced energy expenditure may be associated with PI3K/Akt and STAT3/SOCS3 signaling disruption and Pro-opiomelanocortin expression.

Keywords MiRNA · STAT3 · PI3K/Akt · Oxidative stress

Introduction

Obesity is a complex and multifactorial disease, with high prevalence rates in today's Western societies. It is estimated that 2.1 billion people are overweight or obese in the world

[1]. Excess of body mass can result from the imbalance between energy intake and expenditure, moreover, imbalance in adipose tissue homeostasis, sedentary lifestyle, genetic inheritance, family environment, cultural experiences, previous or current diseases are among other risk factors [2].

In normal conditions, the food intake and energy expenditure are controlled by the arcuate nucleus of the hypothalamus (ARC), where the synthesis and the secretions of orexigenic and anorexigenic neuropeptides occurs. Orexigenic neuropeptides are neuropeptide Y (NPY) and the peptide related to the Agouti gene (AgRP) that stimulates the appetite and decreases energy expenditure. On the other hand, anorexigenic neuropeptides proopiomelanocortin (POMC) and the cocaine and amphetamine-related transcript (CART) reduce food intake and increase energy expenditure. Synthesis and secretion of these neuropeptides (NP) are controlled

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by negative feedback mechanisms, however, hormonal, genetic, or epigenetic modifications can damage their synthesis and secretion, by altering the food intake control system and energy expenditure, resulting in eating disorders which can result in a severe increase of body mass and metabolic diseases [3].

In the last decades, epigenetic modifications have been identified as important mechanisms responsible for the development of several diseases, including obesity and type 2 diabetes mellitus (T2DM). With regards to appetite control, studies have mainly evaluated epigenetic modifications resulting from maternal obesity [4], neonatal feeding, and ingestion restriction of certain nutrients [5]. However, there are still controversies about the effects of epigenetic modifications on the synthesis and secretion of NP regarding food intake. Moreover, the search for epigenetic therapy for diseases such as obesity and T2DM have significant scientific and therapeutic appeal, because of the reversible nature of epigenetic modifications and its link with those pathologies.

In addition, oxidative stress is the result of reactive oxygen species (ROS) overproduction and/or a decline in antioxidant defense mechanisms. Excessive generation of ROS can result in deleterious effects causing damage to DNA, proteins, and lipids structure, ultimately leading to change in cell homeostasis and cell death. Obesity can induce systemic oxidative stress through various biochemical mechanisms, such as superoxide generation from NADPH oxidases, oxidative phosphorylation, and protein kinase C activation. Furthermore, growing evidence suggests the association of oxidative stress as the critical factor linking obesity with its comorbidities [6]. Thus, in this review, we synthesize the state of the art over epigenetic modifications in the neuropeptides, NPY, and POMC, of ARC and its relationships with oxidative stress, the development of obesity, and metabolic diseases.

Long-term feeding control

Obesity is the result of a positive energy balance due to increased high caloric food intake combined with a sedentary lifestyle resulting from frequent exposure of peoples to an obesogenic environment [7]. Nevertheless, the physiological control of food intake and choice of food is regulated via the interaction between energy homeostasis and complex neural circuits in the hypothalamus. In general, signals produced in the digestive system and decoded by sensory receptors in central brain structures can stimulate or inhibit food and energy expenditure [8]. Generally, the control of food intake is divided into two stages: the short-term and the long-term [9].

The short or long-term signals are transmitted to the central nervous system (CNS) and are integrated by the

hypothalamus that regulates the acquisition and expenditure of energy [10]. The short-term control is related to the controls regards to the beginning and end of a meal, while the long one controls the expenditure and the stored energy. The hypothalamus structures such as ARC, the nucleus of the solitary tract, the lateral hypothalamus, and other brain stem nuclei are pointed as structures acting in this control [9]. In ARC, there are two main populations of neurons involved in the regulation of food intake and energy expenditure: neurons that synthesize anorexigenic NP, POMC, and CART; and neurons that synthesize orexigenic NP, NPY, and AgRp [11].

The neuronal ARC populations that synthesize anorexic NP are projected to the paraventricular nucleus (PVN), while orexigenic NP synthesizing neurons are projected to the lateral hypothalamus (LH). Therefore, the balance between the outputs of PVN and HL plays a critical role in the regulation of food intake and energy expenditure [12].

The mechanism of long-term control feeding is mainly carried out by the insulin secretion by pancreatic beta cells after the food intake of carbohydrates and the secretion of adiponectin, including leptin from the adipose tissue. These hormones stimulate the secretion of POMC and inhibit the secretion of orexigenic neuropeptides from ARC [11]. When there are exacerbated release of insulin and leptin, the signaling process is impaired, so that the expression and secretion of orexigenic and anorexigenic NP and food control are damaged, resulting in hyperphagia or anorexia [13]. Through hyperphagia and a reduction in energy expenditure, the accumulation in the body index can result in obesity and related comorbidities.

Leptin decreases food intake and increases energy expenditure through its effects on hypothalamic ARC, mainly by its actions on the long form of the leptin receptor (LEPRb). Leptin binding to the LEPRb activates Janus kinase 2 (JAK2), thereby initiating several transduction signal pathways, as the phosphorylation of activator of transcription 3 (STAT3), an important transcription factor. The subsequent effect is the dimerization and translocation of STAT3 into the nucleus. In the nucleus, STAT3 proteins bind to the promoter and regulate transcription of POMC genes. So the activation of the STAT3 signaling increases POMC neuronal activity in the ARC and its synthesis and secretion, preventing the food intake and regulating the bodyweight [14].

Regarding insulin, the central insulin receptor (IR) is located within hypothalamic ARC and when stimulated by insulin promotes the phosphorylation of IR and of insulin receptor substrate (IRS) 1 and 2, which, in turn, activate phosphoinositide 3-kinase (PI3K) and serine-threonine kinase (Akt) in the ARC. PI3K catalyzes the phosphorylation of the phosphatidylinositol-4,5-bisphosphate (PIP2) and thus generates phosphatidylinositol-3,4,5-trisphosphate

(PIP3). PIP3 can activate ion channels, and phosphatidylinositol-dependent kinase 1 (PDK1), which phosphorylates several proteins such as Akt to elicit signaling events [15]. Phosphorylated AKT migrates to the nucleus and phosphorylates the forkhead transcription factor—FOXO1. Once phosphorylated FOXO1 migrates to the cytoplasm, where it remains inactivated, inhibiting the gene promoter which transcribes POMC [16].

Both insulin and leptin signals converge at the level of PI3K because phosphorylated JAK2 is able to activate the substrate of the IRS, which activates PI3K [17]; leptin and insulin stimulation leads to phosphorylation and nuclear exclusion of the forkhead transcription factor FOXO1, allowing for STAT3 binding to the promoter and transcription of POMC [15, 18]. In addition, Morrison [19] suggest that leptin activation of STAT3 is insufficient to stimulate expression of POMC in the absence of PI3K signaling [19]. So, POMC transcription is stimulated by leptin, that phosphorylates JAK2 and stimulate STAT3, which moves to the nucleus, in which it will activate transcription, or by the PI3k/Akt pathway, which is the common pathway of insulin and leptin (Fig. 1).

Different factors have been proposed to explain the mechanism that underlies hypothalamic leptin and insulin resistance and the resultant change in appetite behavior. However, the epigenetic modifications are recently the main mechanisms supposed to explain the changes in hypothalamic POMC and NPY expressions [11] and will be discussed in this review. So, in the next chapters, are presented the principal epigenetic modifications.

Epigenetic modifications

Physiological structure or function could be altered via activation or silencing for certain genes [20]. Researchers adopted the Greek word epigenesis and changed it to epigenetics to represent changes in gene expression, independent of DNA changes. Recent studies have shown that epigenetic modifications play an important role in many acquired chronic diseases, such as obesity and metabolic diseases, where small changes in the epigenome alter the cell phenotype leading to the manifestation of the epigenetic disease [21]. The three main epigenetic mechanisms described are

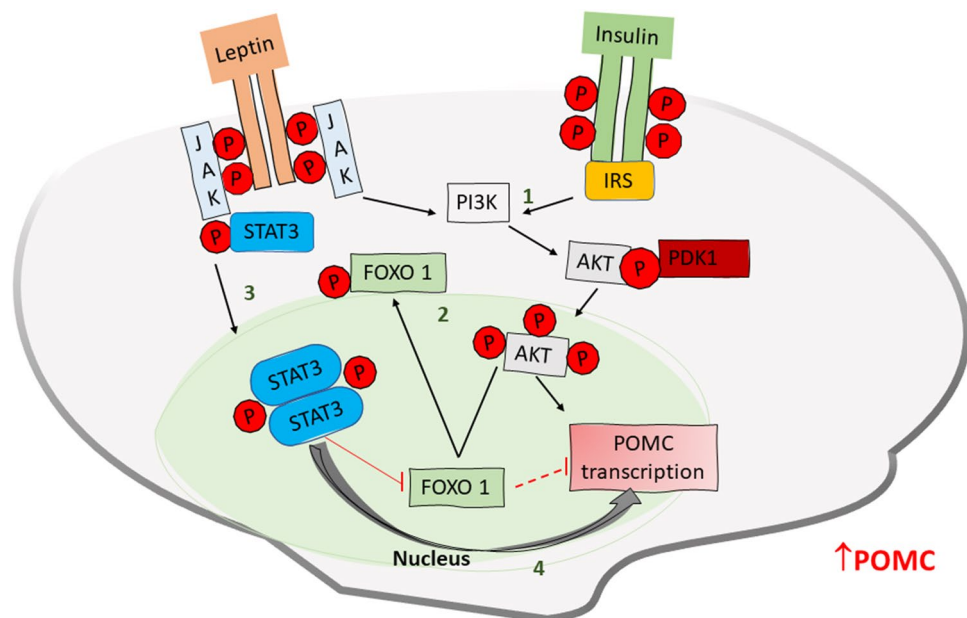


Fig. 1 Insulin and leptin signaling mechanism in the arcuate nucleus. (1) the stimulation of central insulin receptors (IR) stimulates the phosphorylation of IR1 and IR2, which activate PI3K (protein kinase 3) and protein kinase B/Akt. PI3K becomes activated and phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) on position 3' in the inositol ring, generating PIP3. The protein kinase B/Akt (serine-threonine kinase) and phosphoinositide-dependent protein kinase 1 (PDK1) bind to PIP3. When stimulated by PI3K, Phosphoinositide-dependent protein kinase 1 (PDK1) stimulates Akt. (2) Phosphorylated Akt enters the nucleus, where it phosphorylates the forkhead transcription factor (FOXO1) and this leads to exclusion from the

nucleus and thereby to inactivation of FOXO1, a negative regulator of POMC expression. (3) Leptin binds to receptors activating JAK2 (Janus kinase protein 2) which phosphorylates STAT3 (Signal Transducer and Transcription activator 3). Upon phosphorylation, two STATs homodimerize and translocate to the nucleus, where they activate target genes. Leptin also activates PI3K signaling, via JAK2 mediated phosphorylation of IRS proteins. (4) the integration between the action of insulin and leptin is through PI3K because phosphorylated JAK2 is able to activate the substrate of IRS, which activates PI3K. (5) The interaction of the IP3/Akt and STAT3 process activates the transcription of POMC

DNA methylation, microRNA formation, and histone acetylation or methylation, which will be briefly discussed in the following paragraphs.

In the mammalian genome, DNA methylation is a process that corresponds to the addition of a methyl group (CH₃) in the fifth carbon of a cytosine residue thus forming 5-methylcytosine (a process that is facilitated by the action of the DNA methyltransferase enzyme) causes repression of gene expression. This process regulates the gene expression, recruiting proteins involved in the regulation of genes and cell differentiation. Most DNA methylations occur in cytosines that precede guanine or in CpG sites (dinucleotides with a higher density than cytosine and guanine. The binding of the methyl group on the CpG sites in association with the N-terminal histone creates a silenced chromatin structure. Methylation reduces gene expression because it generates a physical barrier that prevents the recognition of transcription factors, inactivates the gene, or attracts proteins such as MeCP2, that recruit histones co-repressants and deacetylases, which inactivates chromatin around the gene [21].

In the process of DNA demethylation, the 5-methylcytosine (5mC) can convert into 5-hydroxymethyl cytosine (5hmC), a reaction that probably promotes gene expression and demethylation. The conversion of 5mC into 5hmC by TET (ten-eleven translocation enzymes), by the repressive DNMT (DNA methyltransferase), and the binding of the domain, contains proteins that would be normally recruited by 5mC. DNMTs and TETs collectively and antagonistically regulate the epigenetic dynamics of methylation [22, 23].

MicroRNAs (miRNAs) are small and single strands, of non-codable RNAs that have 18 to 25 nucleotides, which often bind to untranslated regions of messenger RNAs (mRNA). Among its functions, we can mention the silencing and post-transcriptional regulation of the gene expression of RNA messenger preventing protein translation. The DROSHA enzyme folds the miRNA gene to form a clamp structure called Pri-miRNA, employing the DGCR8 enzyme forms Pri-miRNA, which is released into the cytoplasm. In the cytoplasm, the DICER enzyme breaks up the clamp structure, forming the double strand of miRNA, which is ligated into a single strand of miRNA. DICER and DICER-derived miRNAs are essential for the integrity of leptin signaling in the hypothalamus. On the other hand, these miRNAs also could increase the translation of specific targets. Each tissue exhibits a miRNA expression profile, which suggests specific functions and targets. Its differential expressions can be adjusted by the environment and have been linked to various biological functions and human pathological conditions. They are often described as epigenetic modulators, due to their ability to affect protein levels of target mRNAs, without modifying their DNA structure [24]. Besides, miRNAs are important in the regulation of many cellular and developmental pathways, changing their expression profile in many

diseases, mainly cancer, and cardiovascular diseases. This expression profile has been quite prominent in the prognosis, diagnosis, and treatment of many diseases [25]. It has been shown that in the hypothalamus, adult deletion of DICER in the ARC caused hyperphagia and obesity [26].

Methylation or acetylation of histones are in the chromatin (a highly ordered structure present in the cell nucleus). Histones are proteins that aggregate to form the nucleosome, basic components of the chromatin. The n-terminal tails of histones are subject to post-translational modifications that can influence several biological processes, such as transcription, replication, and maintenance of chromosomes [27]. The histone acetylation describes the transfer of an acetyl-coenzyme A (acetyl-CoA) to the primary amine at the ϵ position of the lysine side chain within a protein, causing neutralization of the positive electrostatic charge of the position. This process is driven by enzymes, metabolites, and cofactors that balances the acetylation levels [28]. Histone methylation contributes to chromatin configurations, however, these configurations depend on the histone to be methylated or acetylated and the modified residue [29]. The results of histone methylation or acetylation involve a more complex process inhibiting or activating expression than CpG methylation of DNA. Methylation of histones typically occurs in lysine (K) or arginine (R) and the results depend on which position the amino acid and the number of methyl groups were added. For example, the monomethylation of histone 3 lysine 9 (H3K9me) and H3K4me promote activation, however, H3K9 di-methylation is generally associated with genetic repression [30, 31].

Epigenetic modifications of NPY and POMC expression in early life phases

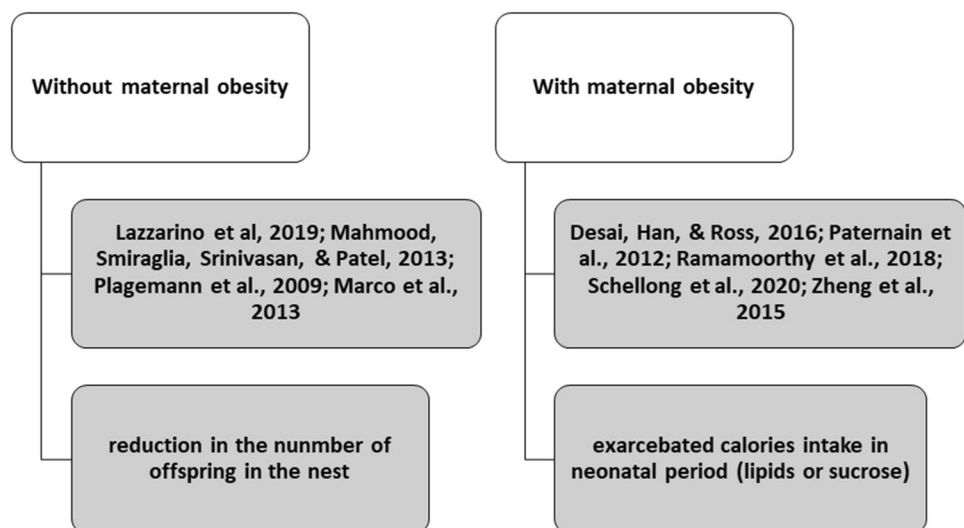
In a previous review of our research group [23], we proposed that epigenetic modifications are caused by enhanced alimentary intake and can cause overfeeding and obesity such as DNA methylation in POMC promoters and protein recruitment of methyl-CpG-binding 2 (MeCP2); histone modifications such as H3K9Ac acetylation and decreased H3K27me3 trimethylation and increasing the micro RNAs formation that promotes decreased POMC expression and increase in NPY. However, most of the studies are focused on the methylation mechanism of the CpG portion of POMC or NPY promoters. Studies published until 2019 mostly evaluate NPY and POMC DNA methylation, on the development of obesity in the neonatal period, due to maternal obesity and overfeeding after birth. Table 1 shows a summary of those studies. To evaluate data about NP expression and methylation of the promoters, we established the comparison between the studies that used similar protocols without maternal obesity and with the induction of body

Table 1 Comparison of changes caused by high-calorie diets or by overeating in the expression of Neuropeptide Y (NPY) and Proopiomelanocortin (POMC) of the hypothalamic arcuate nucleus

| Reference | BW | CI | NPY Expression | NPY Promoters | POMC expression | POMC Promoters |
|----------------------------|--|-----|-------------------------------------|--|---|--|
| 1. Plagemann et al. [34] | ↑ | φ | → | → methylation of CpG from the regulator factor of growing up (NGF-RE; -78 a -36), (NGFI-A; -62) e activating protein 2 (AP-2; -62) | → | ↑ of methylation CpG of nuclear factor κB e SPI |
| 2. Paternain et al. [32] | ↑ | → ↓ | φ | φ | ↑ | ↑ of methylation CpG site -167 pb |
| 3. Mahmood et al. [35] | ↑ | φ ↑ | φ | ↓ of methylation CpG position 21 (gen M15792, localization 2242 to 1103) → of methylation H3K9 | ↓ after 16 days → after 100 days | → of methylation CpG (gen M74296, position 2298 a 143) → methylation H3K9 |
| 4. Marco et al. [37] | ↑ | ↑ φ | φ | φ | → | ↑ Sp1, ↑ of methylation CpG percentage cytokines in the POMC promoter |
| 5. Zheng et al. [38] | ↑ | φ φ | φ φ | φ φ | ↑ high-fat, high-sucrose diet | ↓ of methylation CpG 2,5,6,7 e 8 specific sites promoters of POMC in the base pair fragments 322. No methylation was found on the MC4R promoter of the groups |
| 6. Desai et al. [33] | → in offspring that was born to obese mother, ↑ when fed with breast milk (obese mother) | ↑ φ | φ | φ | → with 1 day age ↓ at 6 months of age due to suppression of Mash 1 and NgN | ↓ in the expression of MASH 1 (necessary for the normal development of POMC) ↓ From DNMT1 (responsible for the methylation) |
| 7. Ramamoorthy et al. [39] | ↑ | ↑ | ↓ in the offspring with 3 weeks age | φ | → mRNA in POMC of HF offspring | ↑ of methylation of CpG sites next from the bind locations SPI |
| 8. Schellong et al. [40] | ↑ pre-pregnancy and during the pregnancy; → offspring | φ φ | φ φ | φ φ | → | ↑ of methylation in unique locations of CpG in females, like PromS1_CpG4 e PromS1_CpG5 ↑ of methylation of V2S1_CpG1, V1S1_CpG2, V1S1_CpG3 → Short-term regulatory sequences |
| 9. Lazzarino et al. [36] | ↑ | ↑ → | → | ↓ of methylation of BstUI (a) e BstUI (b) mid and long term | ↑ | ↓ Methylation of POMC promoter in Mae II and BstUI sites ↑ mRNA POMC Long-term |

NPY neuropeptide Y, AgRP Agouti-related peptide, POMC proopiomelanocortin, CART cocaine and amphetamine-related transcript, MBI body mass index, CI caloric intake, ↑ = increase, ↓ = reduction, → = no changes in the expression, φ not evaluated, BW body weight

Fig. 2 Summary of articles and respective methodology used to research Neuropeptide Y (NPY) and Proopimelanocortin (POMC) methylation and its effect on obesity in offspring



mass increase only in the offspring (articles 1,3 and 8 of Table 1) or associated with maternal obesity (articles 2,4,5,6 and 7 of Table 1) (Fig. 2).

In general, rodents are the most used animal model, and species used were Wistar rats, Sprague Dawley, and guinea pigs. The basic protocols used are described in Fig. 2. In the analysis of studies in this present review (Table 1), the protocols used to induce an increase in body mass were efficient and caloric intake, when evaluated, was higher in animals submitted to increase calorie supply compared to controls. The exception was in Paternain et al. [32], which induced the stress response in animals to promotes maternal undernutrition. Such as maternal obesity, it has been shown that maternal undernutrition results in offspring with a marked predisposition to the development of metabolic syndrome, including obesity, hypertension, and diabetes [33].

Comparing results of the articles that used rodents without maternal obesity [34–37] expression of NP and promoters of methylation presented divergent results. Plagemann et al. [34] did not observe changes in NPY methylation or expression however there was an increase in POMC promoter methylation, which was not accompanied by the decrease in NPY expression. In POMC there was hypermethylation in Sp1 and Nf-kB promoters, which are essential for mediates the effects of leptin and insulin; and in signaling to the ARC for POMC expression. Marco et al. [37] examined expression levels of Sp1 transcription factors. The High-Fat group displayed significantly Sp1 (signal transduction pathway, which is essential for the mediation of leptin effects on POMC expression) and POMC CpG methylation percentage at sites 7, 11, and 11r were higher, both at the mRNA and protein levels, compared to the control group. However, they showed no change in POMC expression levels, despite the sharp increase in leptin and insulin [37].

Mahmood et al. [35] observed hypomethylation and increased NPY expression; without altering POMC methylation but with reduced expression. The H3K9 dimethyl levels of the NPY and POMC genes were also assessed and, there were no differences in H3K9 methylation or dimethylation between groups [35]. On the other hand, Lazzarino et al. [36] found different patterns from Plagemann et al. [34] and Mahmood et al. [35] in the expression of the orexigenic peptides from the lateral hypothalamus. The POMC expression was higher in the last experimental week in the group fed with a balanced diet for rats, while NPY expression was lower in both groups in the twentieth week compared with the first experimental week. Methylation of POMC and NPY promoters decreased from the middle of the experiment to the end in both groups.

When maternal obesity was assessed, there was also no consensus among the studies concerning the results of methylation and expression of NPY and POMC. As discussed, an increase in body mass was found in all studies when compared to animals in the control groups, together with metabolic changes similar to T2DM and metabolic syndrome. Paternain et al [32] found an association between hyperinsulinemia and hyperleptinemia and increased methylation of POMC promoter, however without an increase in POMC expression. Another interesting result was that animals in the high-calorie diet group had increased methylation of the CpG islands of the active dopamine transporter Slc6a3. This change means that, in addition to the lower inhibition of food intake, there was also an increased feeling of pleasure due to the intake of a high-calorie diet [32].

In the study of Zheng et al. [38] the POMC gene expression increased significantly in the offspring exposed to a high-calorie diet during pregnancy, lactation, and at the age of 32 weeks, while in the hypothalamus there was a hypomethylation of the POMC promoter [38].

Interestingly, Desai et al [33] found that, even with moms that were obese, puppies were born with body mass similar to the puppies of the control group. However, if these puppies were fed by their mothers, their fat index increased markedly. POMC expression remained the same at one day of age, however, a decrease in MASH 1, a promoter of POMC, caused an increase in AgRP expression and a decrease in POMC expression. These results lead to an increase in caloric intake and a decrease in energy expenditure in these animals.

On the other hand, Ramamoorthy et al. [39] tested the effect of the administration of a high-fat diet for 6 weeks, in males and females before mating, during the pregnancy, and during the lactation period. Despite the increase in methylation of POMC promoter genes, POMC RNA expression remained the same in the offspring. In contrast, Schellong et al. [40] observed changes in the methylation pattern of the POMC promoter (both in males and females), whereas in the methylation pattern of the AgRP promoter there was a significant decrease only in male puppies. Thus, the epigenetic changes that occurred in the offspring of obese mothers are the result of the heritability of these modifications, but, in the overfed offspring in the

initial phase of life, the epigenetic changes must be due to metabolic changes that emerged before obesity.

Oxidative stress, PI3K/Akt/PIP3, and JAK2/STAT3/SOC3

It is well recognized the effect of diet, namely the hyperlipidic/hypercaloric, mainly high fat diet (HFD), food intake in the process of generating oxidative stress and inflammatory factors [41, 42]. Due to the ingestion of this type of diet occurs the production of reactive oxygen species (ROS), and this prompts transcriptional activation of proinflammatory cytokines (ex. IL-1, IL-6, TNF- α). Besides, the overeating and/or the ingestion of hyperlipidic foods increases the plasma concentration of fatty acid (AG), the high level of AG induces the infiltration and activation of macrophages in adipose tissue, causing tissue inflammation, which reinforces the secretion process of pro-inflammatory factors, reaching the systemic circulation [43], impairing the cell process by disrupting PI3K-Akt-mammalian target of rapamycin (mTOR), a serine/threonine protein kinase of the PI3K, and STAT3 signaling (shown in Fig. 3).

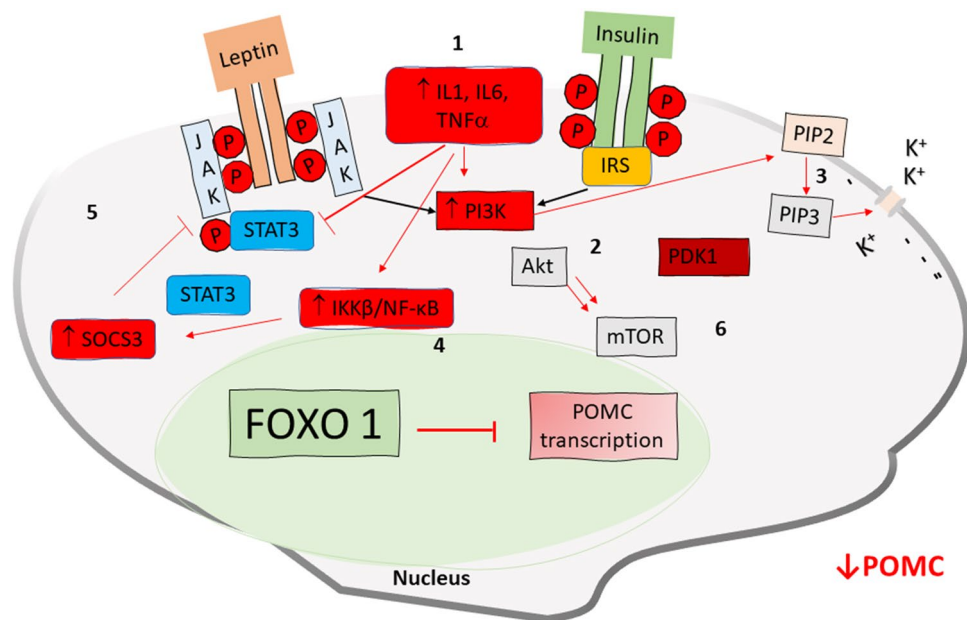


Fig. 3 (1) Proinflammatory cytokines and cell signaling: the intake of hypercaloric/ hyperlipidic foods increases the plasma concentration of fatty acids and proinflammatory cytokines, inducing the activation and infiltration of macrophages in adipose tissue, causing tissue inflammation. (2) oxidative stress (ROS) and pro-inflammatory cytokines increase PI3K/Akt pathway activity converting Phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). Increased PIP3 open ATP-sensitive potassium (KATP) channels (K⁺/ATP) with consequent hyperpolarization and inhibition of POMC secretion. (3) The downregulation by PI3K/

PIP3-effect is important in the process of regulating the set point of POMC secretion, nevertheless, chronic activation of PI3K/PIP3 leads to leptin resistance. (4) The impairment of signaling will result in the inhibition of leptin-induced tyrosine phosphorylation of STAT3, the inhibitor of the forkhead transcription factor (FOXO1). (5) Enhanced IKK β /NF- κ B (inflammatory response) up-regulates the expression of suppressor of cytokine signaling 3 (SOCS3), a negative regulator of leptin signaling; (6) over-activation of mTOR by Akt causes an imbalance in the levels of anorexigenic and orexigenic neuropeptides, resulting in hyperphagia and obesity

In a physiologically situation, activation of the PI3K-Akt-mTOR pathway in hypothalamic neurons reduces food intake as a reaction to the availability of nutrients. However, the regulation of PI3K-Akt-mTOR activity in response to overfeeding can vary depending on the type of cell and the region of the brain examined. In fact, abnormal chronic overactivation of PI3K-Akt-mTOR in the hypothalamus has been shown to lead to obesity, even without increasing food intake, that is, by reducing energy expenditure [26]. Hyperactivation of mTOR is often associated with inflammation and dietary restriction reduces mTORC1 activity in some mammalian tissues, and is sufficient to extend lifespan in mice. In peripheral metabolic tissues, such as muscle and liver, during nutritional excess in high-calorie feeding and obesity also contributes to insulin resistance and metabolic dysfunction [44].

Regardless of the activation of the mTOR pathway, Plum [45] demonstrated a marked increase in the K_{ATP} current of POMC neurons, supporting the hypothesis that electrical silencing of POMC neurons results from PIP_3 -mediated activation of K_{ATP} channels. Thus increase in PIP_3 formation lead to activation of K_{ATP} channels and subsequent silencing of POMC neurons [45]. In this way, it is valid to assume that the ROS and pro-inflammatory cytokines can over-activate PI3K, increase PIP_3 and K_{ATP} channel activation in unidentified hypothalamic neurons with reduced electrical activity and POMC secretion, resulting in hyperphagia and obesity.

Concerning JAK2/STAT3 signaling, it has been shown that enhanced expressions of interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha suppress the inhibition of cytokine signaling 3 (SOCS3), a recognized inhibitor of insulin and leptin signaling [46]. The overnutrition or high fat diet-induced-activation of IKKbeta/nuclear factor-kappa B (NF-kappaB) is a key of intracellular inflammatory pathway, particularly in hypothalamus [47]. Despite IKK β is present in neurons of the ARC, its signaling remains suppressed under normal nutritional conditions, however, overnutrition or high-fat feeding, ROS enhance IKK β /NF- κ B signaling in the hypothalamus, impairing Leptin signaling [46]. Enhanced IKK β /NF- κ B increases the expression of SOCS3, a natural negative feedback mediator of leptin signaling, resulting in leptin and insulin resistance in the CNS. SOCS3 inhibits JAK2-STAT3 signaling through binding to a specific tyrosine residue on LEPRb [15]. Therefore, the impairment of signaling will result in inhibition of leptin-induced tyrosine phosphorylation of STAT3, the inhibitor of FOXO1, and activator of POMC expression, decreasing the POMC transcription and its effects in reducing food intake (Fig. 3).

PI3K/Akt, STAT3/SOC3 and POMC expression

Table 1 shows several experimental reports of epigenetic changes caused by exacerbated food and/or caloric intake, with the exception of Mahmood et al. [35] and Marco et al. [37]. Despite they evaluated the relationship between food and epigenetic changes, they do not link with the signaling performed via PI3K/Akt and STAT/SOC3. Still, they provide evidence of the effects of diet on DNA methylation and changes in the expression of genes related to the control of food intake and energy expenditure.

The link between PI3K/Akt and STAT/SOC3 pathways in miRNA and POMC control was shown by Derghal et.al [48]. Through an *in vitro* study the research group investigated the pathways of Leptin in miRNAs expression using Hypothalamus cell treated with final concentrations of leptin in culture medium if 1, 2.5, or 5 μ M for 1.5 h or 3 h. The control group cells received vehicle alone. They were able to demonstrate that Leptin modulates the expression of miR-383, miR384-3p, and miR-488 by different pathways: the JAK2-STAT3 pathway is involved in the regulation of miR-384-3p and miR-488 by, while miR-383 is regulated via PI3K-Akt pathway. miR-383 and 384-3p targeting the expression of POMC and play predominantly inhibitory regulatory roles by binding to cis-element in the 3' untranslated region (3'UTR) of message-encoding RNAs. Besides, they demonstrated that 5 μ M of leptin treatment in mHypoA-POMC/GFP culture for 2 h significantly decreased miR-383, miR-384-3p, and miR488 levels (Fig. 4A) [48]. In cancer studies, has been suggested that miR-383 suppressed the PI3K-AKT-mTOR signaling pathway to inhibit development of cancer via down-regulating Poly (ADP-ribose) polymerase-2 [49]. In this way, it is reasonable to suppose that due to hyperleptinemia, mir-383 reduces and PI3K-AKT be overactivated, so the expression of POMC be reduced.

Concerning miRNAs, DICER and DROSHA are the key endoribonucleases responsible for microRNA biogenesis, moreover, only DICER reacts to decreased nutrient availability in the hypothalamus during fasting and its expression decreases in diet-induced obesity [31, 50]. Vinikkov et al. [26] demonstrated that by removal of DICER the reduction of microRNAs, namely miR-103, from ARC neurons leads to severe hyperphagia and obesity on the regular chow diet and that this phenotype critically depends on chronic over-activation of the PI3K-Akt-mTOR pathway. It is worth mentioning that the normal activation of PI3K-Akt, mTOR by insulin induces a reduction in food intake, however, this over-activation caused an imbalance in the levels of anorexigenic and orexigenic neuropeptides, resulting in severe hyperphagic obesity [44, 50]. Besides, DICER deletion occurred early in development, severely affecting proper differentiation of POMC cells leading to the development

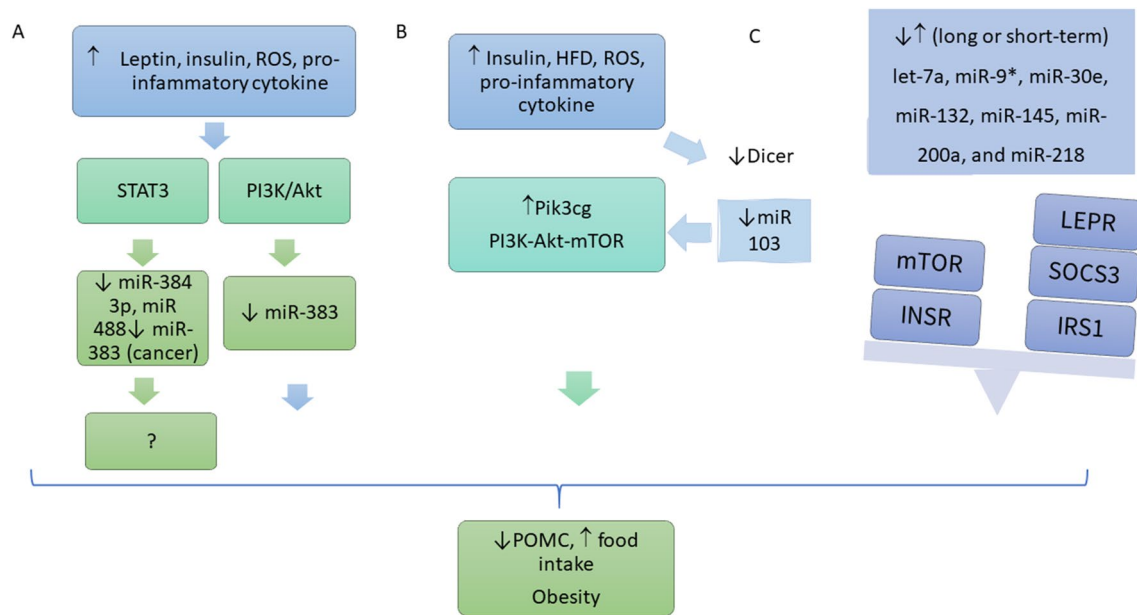


Fig. 4 **B** Increased Insulin, HFD (high fat food), ROS (reactive species of oxygen) and pro-inflammatory cytokine induces to reduction of DICER, the enzyme responsible for generating miRNA, so reduced mir103 contributes to phosphatidylinositol 3-kinase/serine threonine protein kinase/ mammalian target of rapamycin (PI3K-Akt-mTOR) pathway overactivation and proopiomelanocortin (POMC) expression reduction; **C** depending on short or long term overfeeding or hypercaloric food intake, reduction or increase in specific micro RNA (miRNA) causes disturbances in the insulin, leptin and mTOR sign-

aling pathways; **A** Increased Leptin, insulin, ROS, pro-inflammatory cytokine overstimulate Janus kinase 2/signal transducer and activator of transcription (STAT)3 (JAK2-STAT3) pathway regulation of miR-384-3p and miR-488 by leptin while exacerbated leptin and insulin via PI3K-Akt pathway reduces miR-383, in this way, over-activation of mTOR by Akt causes an imbalance in the levels of anorexigenic and orexigenic neuropeptides, resulting in severe hyperphagic obesity. The role of mi-384 3p and mi-488 in the expression of POMC are still controversial

of obesity [51], demonstrating that this balance in the formation of microRNAs is crucial for the correct maintenance of POMC expression (Fig. 4B,C).

Moreover, the authors suggest that upregulation of the Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform (Pik3cg) transcript in response to the loss of miR-103 in ARC may have contributed to PI3K-Akt-mTOR pathway overactivation and obesity (Fig. 4B). Furthermore, the authors propose that a detailed delineation of microRNA–target interactions, such as miR-103 and Pik3cg in a specific cellular context within the hypothalamus, is of huge importance for the understanding of control mediated by ARC on food intake and energy expenditure [26]. Additionally, Sangiao-Alvarellos et al. [52] showed that diet (HFD) administration altered the expression of 74 out of 641 miRNAs analyzed, including let-7a, miR-9*, miR-30e, miR-132, miR-145, miR-200a, and miR-218 (Fig. 4C). The target predictions, performed by simulation of algorithms, were the components of signaling pathways, already pointed out in the present review, such as NF- κ B, interleukins, PI3K/AKT, ceramides, insulin receptor, p70S6K and JAK/STAT [52]. Paradoxically, despite the reported reduction of DICER in studies with HFD, the assays for 2 weeks or one month of mir-145 and mir-218 miRNA showed that levels reduced

at initial procedures and increased after 1 month, while hypothalamic let-7a and mir-9* expression levels were consistently elevated by the HFD and mir-132 expression was enhanced only at 3 months after the HFD. In contrast, mir-200a expression in the hypothalamus decreased in animals fed with the HFD for 2 weeks and 1 month, but its miRNA levels returned to control values at 3 months after HFD exposure. Following the authors, during the initial stage, mir-30e, mir-145, mir-200a, and mir-218 showed a decline in their expression levels and displayed an increase at later periods (mir-218, mir-145, and mir-30e) as a compensatory response to restore homeostasis against the metabolic deregulation that occurs when obesity is installed. The consequences of miRNA changes were disturbances in the insulin, leptin and mTOR signaling pathways and inflammation signalling (increased NF- κ B) [52].

Regarding to DNA methylation (Table 1), [32, 34, 37, 39] it was found an increased DNA methylation and [40] increased CpG DNA methylation of POMC, however they found no reduction in the expression of POMC. On the other way, Mahmood [35] found reduced POMC expression, without alteration of DNA methylation, and the investigation of histone tail modifications on hypothalamic chromatin extracts from 16-day-old rats indicated decreased

acetylation of lysine 9 in histone 3 (H3K9) for the POMC gene [35]. Although these studies have evaluated Dna and histone methylation, they have not evaluated relationships with PI3K/Akt/mTOR or with JAK/STAT3, but Desai et al. [33] induced Sprague Dawley to maternal obesity, using a high fat diet prior to and through pregnancy and lactation, and determined the protein expression of mTOR and pAMPK energy sensors, epigenetic factors as DNA methylase, DNMT1, histone deacetylase, SIRT1/HDAC1 (an NAD⁺-dependent histone deacetylase), AgRP and POMC in newborn hypothalamus and adult arcuate nucleus [33].

As a consequence of the maternal HFD, Desai et al [33] found the following results: dams were heavier than controls, however, despite maternal obesity, male offspring born to obese dams had similar body weight at birth. Nevertheless, when nursed by the same dams, adult male offspring of obese dams demonstrated marked increased body weight, adiposity and hyperphagia. Although the changes presented at 1 day of age, as reduced expression of DNA methylase DNMT1 and decreased expression of energy sensors mTOR, at 6 months of age HFD adult males showed significantly increased ARC expression of mTOR/AMPK and normalized expression of the DNMT1. Furthermore, ARC expression of SIRT1 and HDAC1 was significantly suppressed in HFD and the POMC expression was decreased when compared to Control males.

Regarding reduced SIRT1/HDAC1 expression, SIRT1 interacts physically with HDAC1 and promotes histone deacetylation. In this way, the study failed to establish whether histone-mediated suppression of gene transcription occurs in the HFD offspring because whereas histone acetylation at specific loci correlates with open chromatin state permitting gene transcription, deacetylation effectively suppresses gene transcription. Concerning DNMT1, 6 months old HFD newborns demonstrated similar expression in the ARC as the Controls. It is worth mentioning that DNMT1 is responsible for maintaining DNA methylation and reduces the expression of genes sensitive to promoter CpG methylation levels, in fact, the protocol was also not able to demonstrate an effect in reducing POMC, despite the increase in the weight of the HFD animals. In contrast, mTOR and AMPK (a protein kinase which elicits cellular response to acquire energy sources and reduce energy expenditure) expression were increased, a consistent result with the increased food intake and weight gain [33]. So the mechanism by which DNMT1 and histones modifications regulate POMC expression in response to HFD and promote offspring hyperphagia and obesity remains controversial and requires more studies.

Putting all together, during chronic inflammation, defects in insulin and leptin signaling may be responsible for epigenetic modifications in gene expression of POMC, and that these effects may be linked to changes occurring

in the PI3K/Akt and/or STAT3/SOC3 pathways. In turn, increased PI3K/Akt may cause microRNA production/reduction (Fig. 4) disrupting the regulation of POMC expression. Although the effect of oxidative stress and pro-inflammatory cytokines on increased DNA methylation and histones regulation of POMC expression still waits for more investigation. Additionally, several studies concerning cancer development have been pointed out PI3K/Akt and/or STAT3/SOC3 pathway deregulation as important factors to epigenetics modifications [53–55]. So, we suppose that these signaling pathways may be the focal point of studies related to the effect of oxidative stress on epigenetic modifications on POMC control of caloric intake.

Conclusion

Specifics reduction or increase in the expression of POMC or NPY and the epigenetic modifications seems to be due to the duration of the experimental procedure, probably resulting from the negative feedback mechanisms and/or the resistance of the receptors to the signals for synthesis and secretion of NP. Unfortunately, the studies evaluated in the present study were done with different genes/situs/locus or different epigenetic mechanisms in different time life of animals, and in this way, we were not able to be found one mechanistic process to better understand the effect of overfeeding on epigenetic modifications. Despite the exposure, in all analyzed papers, the overfeeding in early time-life induced obesity.

Besides, most current research evaluates only the effects of maternal obesity or overfeeding on offspring and in the neonatal and young period, but not during all ontogenetic development in adulthood and aging. Thus, we considered it important to develop studies that assess the effects of exacerbated food intake on the development of obesity and T2DM also in the post-young phase, since it is mainly from this stage, onwards that humans, even without the history of obese mothers or obesity in the neonatal period. Finally, the exacerbated caloric intake can be responsible for the increased oxidative stress, which in turn impairs the signaling process of insulin and leptin, via changes in PIK3/Akt and/or STAT3/SOCS3, resulting in epigenetic modifications and reduced POMC expression. Besides, the most well-described changes so far are changes in the reduction or increase in the formation of specific micro-RNAs.

Author contribution SABR: proposal planning, supervision, manuscript writing, evaluation, and final approval of the manuscript. VALR: manuscript writing, and final approval of the manuscript. MRFM: supervision, and final approval of the manuscript.

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Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Research involving human participants and/or animals This article does not contain studies with human or animal participants carried out by any of the authors.

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