The Association of Short-Chain Fatty Acids and Leptin Metabolism: A Systematic Review

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THESIS

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This thesis is dedicated to my grandparents, who I know are very proud of me for this accomplishment. I carry you with me.

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LIST OF ABBREVIATIONS

AMPK	Adenosine monophosphate (AMP)-activated protein kinase
ARC	Arcuate Nucleus of the hypothalamus
BBB	Blood-brain barrier
CCK	Cholecystokinin
FFAR	Free fatty acids receptors
GLP-1	Glucagon-like peptide 1
GPCR / GPR	G protein-coupled receptors
HFD	High-fat diets
IGF-1	Insulin-like growth factor-1
JAK2	Janus kinase 2
LEPR	Leptin receptor
NPY	Neuropeptide Y
PKA	Protein kinase A
PPAR- γ	Peroxisome proliferator-activated receptor gamma
PTX	Pertussis toxin
PYY	Peptide YY
RS	Resistant starch
SCFA	Short-chain fatty acids
SHP2	Protein Tyrosine Phosphatase-2
SOCS3	Suppressor of Cytokine Signaling-3
STAT	Signal Transducer and Activator of Transcription
Tyr	Tyrosine domains

SUMMARY

Leptin is an adipokine, which can activate a chain of reactions in the hypothalamus to exert its functions. Extreme variations of leptin levels are associated with body dysfunction, which can be influenced by several factors. Nutritional factors, such as short-chain fatty acids have also been related to leptin metabolism through activation of free-fatty-acids receptors 2 and 3. Hence, a comprehensive systematic review to integrate the existing information about leptin and SCFA is important.

This systematic search found 573 articles, which were assessed based on inclusion and exclusion criteria. This led to 36 included articles published between 2002 and 2018: most studies were performed in animals (n = 24), followed by in vitro studies (n = 8) and human studies (n = 4). *In vitro* studies showed that SCFA stimulate leptin expression in adipocytes through activation of FFAR3, but there are no confounding factors. Animal studies represent a realistic approach, considering the complex metabolism that leptin and SCFA have in the body. The interventions to modulate HFD outcomes mostly caused a decrease in leptin concentration and increased in SCFA, added to suppressed body weight – which seems to be the most relevant factor that impacts leptin production. Moreover, SCFA and leptin levels might be related through different pathways, such as AMPK, PKA and PPAR-γ. The improvement in the quality of the microbiota can also play a role in this association. Human studies had very diverse sample and methods, though they also show that leptin is mainly correlated with adiposity, but microbiota also takes part in it.

The current systematic review shows that the association between SCFA and leptin is not completely clear but occurs mainly through the activation of FFAR3 in adipocytes. Leptin expression depends on many factors, but mainly body fat, which is predominant comparing to other influences, such as FFAR3 activation. Interventions that improve dysbiosis and balance SCFA production can have positive outcomes on leptin levels.

INTRODUCTION

A. Statement of the Problem

Leptin is an adipokine, which means it is a hormone synthetized by the white adipose tissue. Its main target is the hypothalamus, where it can activate a chain of reactions to exert its functions such as promote satiety, increase energy expenditure, regulate thyroid hormones, nutrient metabolism, bone metabolism and immunity.[1,2] Extreme variations of leptin levels are associated with body dysfunction. For example, hypoleptinemia is associated with amenorrhea and sleep deprivation.[3,4] On the other hand, hyperleptinemia can trigger leptin resistance, when leptin signaling is impaired, a state that is usually associated with obesity, hyperphagia, inflammation, hyperglycemia and challenges getting pregnant.[5-8] Hence, it is important to maintain adequate leptin concentration and function.

Studies have verified that several factors, such as glucocorticoids, pregnancy, inflammation, caloric restriction, cold exposure and norepinephrine, can influence leptin metabolism. Beyond those, nutritional factors such as prebiotics, probiotics, triglycerides and fructose can also impact leptin synthesis and signaling.[2,6,9-14] Short-chain fatty acids (SCFA), intestinal metabolites with the ability to affect adipose tissue and nutrient metabolism, have also been related to leptin metabolism through activation of free-fatty-acids receptors (FFAR2 and FFAR3).[14] Yet, the association between SCFA and leptin has not been fully studied.

B. Review of the literature

1. Leptin

Leptin is a 167-aminoacid peptide, the product of the *lep* gene, which is located on chromosome 7. Leptin belongs to the family of long-chain helical cytokines due to its crystal structure.[13] There is a 65% similarity in leptin sequence among different species, such as human, gorilla, chimpanzee, dog, cow, pig, rat and mouse.[10]

Leptin was originally discovered in 1995 by cloning the gene in ob/ob mice, a mouse model of obesity, which had a homozygous mutation of the *lep* gene resulting in leptin deficiency. This absence caused hyperphagia, obesity, diabetes, neuroendocrine abnormalities and infertility. That discovery generated excitement based on the belief that leptin could treat obesity, which led to the name of this hormone being derived from the word "*leptos*" that means thin in Greek. However, following studies caused disappointment, as leptin was ineffective for the treatment of obesity – as it will be further explained [section 1d. Leptin resistance].[6]

a. Leptin synthesis and secretion

Leptin is mainly synthetized in the white adipose tissue, which is mainly known for its function as the site of energy storage as fat but is also an important endocrine tissue that produces hormones known as adipokines. Therefore, leptin is an adipokine released in a continuous manner to the extracellular medium, while the intracellular leptin remains constant for a long-term balance. This means leptin is secreted in a continuous, but slow pattern. Leptin secretion has a pulsatile pattern that follows the circadian rhythm, with higher levels at night and early morning.[1,6,11] Leptin is also expressed outside adipose tissue, such as in the bone marrow, placenta, stomach and lymphoid tissue, but the role of this locally produced leptin is still unknown.[15]

b. Leptin signaling

Leptin's structure makes it hydrophobic in some parts, which plays an important role in the bond with receptors.[10] All six leptin receptors (LEPRa to f) are produced by a single Lepr gene and share the same N-terminal site responsible for the bond with leptin. Due to alternative mRNA splicing, these isoforms are different in their C-terminal intracellular domains, which divide them into three categories: the long main receptor (b); short transmembrane receptors (a, c, d and f); and a secreted receptor, which

binds to circulating leptin (e). Leptin receptors are expressed in different cells, tissues and organs, including skeletal muscle, heart, adrenals, kidneys, liver, adipose tissue, immune, pancreatic and brain cells. Yet, the main leptin target is the arcuate nucleus (ARC) and other nuclei of the mediobasal hypothalamus.[13,15]

To exert its biological functions leptin needs to cross the blood-brain barrier (BBB), which does not occur passively, as leptin is too large. This transport happens by a regulated and saturable transport system that is independent of LEPRb. Mainly LEPRa and LEPRc are present in the BBB and transport leptin from the circulation into the brain. Studies show that ARC neurons are strategically positioned to respond to circulating signals, such as leptin. Tanycytes are another type of cells that can also transport leptin into the cerebrospinal fluid, a special type of ependymal cells found in the third ventricle of the brain.[9,13,16]

As LEPRb is the only leptin receptor with a full-length intracellular domain, it is the main mediator of leptin signaling. Although LEPRb does not contain intrinsic enzymatic activity, it can bind to a cytoplasmic tyrosine kinase called Janus kinase 2 (JAK2) and activate it. This bond causes the phosphorylation of LEPRb itself in tyrosine domains (Tyr985, Tyr1077, and Tyr1138) that stimulates three different signaling pathways: [10,13,15]

- Tyr1138 activates Signal Transducer and Activator of Transcription-3 (STAT3). This pathway is responsible for leptin´s main roles.
- Tyr985 activates Protein Tyrosine Phosphatase-2 (SHP2) and Mitogen Activated Protein Kinases;
- Tyr1077 activates Signal Transducer and Activator of Transcription-5.

These pathways are mainly controlled by Suppressor of Cytokine Signaling-3 (SOCS3), a protein that can inhibit leptin signaling through a negative feedback loop. This means that this protein can bind to tyrosine kinase receptors and inhibit signal transduction, blocking leptin´s action through the inactivation

of JAK2. Two of the pathways above are responsible for this feedback, as the activation of STAT3 and SHP2 can lead to SOCS3 activation.[13,17]

c. Leptin function

Leptin has a complex physiological role, but its main function as an endocrine hormone is to control food intake and energy homeostasis.[10] Leptin binding to LEPRb activates ARC neurons, which express the anorexigenic neuropeptides Pro-Opiomelanocortin and Cocaine-and Amphetamine-Regulated Transcript, as well as inhibits the activity of neurons that express the orexigenic neuropeptides Agouti-related Protein and Neuropeptide Y (NPY). Leptin is also responsible for decreasing the activity of lateral hypothalamic neurons, which express melanin-concentrating hormone, which also has orexigenic activity. This same part of the hypothalamus contains neurons that innervate the ventral tegmental area, related to the mesolimbic dopamine system, where the motivation and reward of feeding can also contribute to satiety. Lastly, recent studies have related leptin with the suppression of adenosine monophosphate-activated protein kinase (AMPK) in the hypothalamus, which reduces food intake. Therefore, the overall activity of leptin is to stimulate satiety and suppress appetite.[2,6,10,15,18] In addition, leptin can increase energy expenditure through sympathetic nerve activity. This means that, after binding with LEPRb, leptin can increase uncoupling protein expression, stimulating brown adipose tissue thermogenesis.[2]

During periods of energy deprivation, such as fasting, leptin levels fall rapidly, which can trigger a neuroendocrine response that can decrease reproductive hormone levels to prevent pregnancy, thyroid hormone levels to slow metabolic rate and insulin-like growth factor-1 (IGF-1) to slow growth-related processes, as well as increase growth hormone to mobilize energy stores.[2,6] Leptin also participates in nutrient metabolism. Regarding glucose, leptin takes part in the regulation of glycemia by suppressing

glucagon and improving insulin sensitivity.[2] As for lipid metabolism, leptin stimulates lipolysis and inhibits de novo lipogenesis.[2,10]

Leptin also participates in bone metabolism. It has been shown that leptin can stimulate cortical bone formation and reabsorption trough β-adrenergic stimulation, IGF-1 effects on bone remodeling and inhibition of NPY. Peripherally, leptin also acts on marrow stromal cells to improve osteoblast differentiation.[2] Leptin even plays a role in the immune system as an innate and adaptive immune regulator, due to its similar structure to other cytokines. Leptin participates in hematopoiesis, especially by stimulating the proliferation of leukocytes, such as T-lymphocytes and myelocytic progenitor cells. Leptin also improves macrophage phagocytosis and pro-inflammatory cytokine productions, including tumor necrosis factor-α, interleukins 6 and 12. Taken together, this suggests that leptin has a possible role in controlling inflammation.[2,4,9]

d. Leptin resistance

Circulating leptin levels positively correlate with body fat mass, which leads to overweight and obese individuals to have high leptin concentrations. However, this abundance of leptin fails to restore food intake and adiposity to normal levels, a condition known as “leptin resistance”. Therefore, hyperleptinemia often coexists with a reduced responsiveness to leptin. Although it is still unknown if genetics and/or lifestyle contribute to leptin resistance, it is clear that different mechanisms can limit leptin’s action.[1,19]

Hyperleptinemia initializes the feedback loop that activates SOCS3, which can inhibit JAK2 kinase activity and thus suppress LEPRb signaling. Beyond that, leptin transport through the BBB is impaired in obesity, usually characterized by high-fat diets (HFD), that can impair leptin transport through increased hypothalamic SOCS3 and blood levels of triglycerides.[12,15] Evidence also shows that endoplasmic

reticulum stress and inflammation are activated in obesity states, which can also lead to impaired LEPRb signaling and to reduced leptin transport across the BBB.[1,15,19]

In leptin resistance, there is reduced satiety and hyperphagia, which contribute to obesity maintenance (2). Moreover, a defect in leptin action can contribute to muscle loss through inflammatory pathways. In addition, a dysregulation of lipid and glucose metabolism secondary to reduced leptin activity can contribute to hyperglycemia, hyperinsulinemia and hyperlipidemia.[8]

e. Factors that affect leptin synthesis and signaling

Leptin expression and signaling are regulated by many factors, including hormones and dietary factors, which were all included in Table I.

TABLE I

MAIN FACTORS THAT AFFECT LEPTIN EXPRESSION AND SIGNALING

Stimulate leptin´s synthesis or signaling	Inhibit leptin´s synthesis or signaling
• Adiposity positively correlates with leptin levels;[6] • Glucocorticoids act directly on adipocytes to stimulate leptin synthesis and secretion;[10] • Prostaglandin E2 and arachidonic acid stimulate leptin release by adipose tissue; [10] • Insulin increases leptin synthesis and secretion – the two hormones often act together;[2] • Pregnancy increases leptin levels, which are restored weeks after delivery;[9] • Inflammation and infection increase adipose tissue leptin mRNA and plasma leptin, probably as a response to the immune system production of cytokines;[9] • Prebiotics and probiotics can enhance leptin sensitivity at the BBB, decreasing the harm of HFD;[20] • Free fatty acids receptors: FFAR3 activation stimulates leptin expression, therefore increasing circulating levels.[14]	• Caloric restriction or fasting decrease leptin levels in adipose tissue and in plasma;[2;6] • Cold exposure decreases leptin levels; [11] • Norepinephrine released by the sympathetic nervous system can decrease leptin gene expression; [13] • Beta-adrenergic receptors when activated can decrease leptin expression; [13] • Cyclic AMP inhibits leptin release. [10] • Valproic acid: a psychiatric medicine that can inhibit leptin mRNA levels and secretion; [10] • Dysbiosis: an altered microbiota can lead to impaired leptin signaling in the brain;[14] • Serum triglycerides can inhibit BBB leptin signaling;[12,21] • Long-term high-fructose diet: related to impaired leptin responsiveness in the brain.[12,22.23]

f. Leptin´s correlation with other satiety hormones

Leptin is not the only hormone that suppresses appetite. There are hormones produced by the intestine that signal satiety such as cholecystokinin (CCK), Peptide YY (PYY) and glucagon-like peptide 1 (GLP-1).[24] These hormones are responsible for medium-term satiety, since they are released as the food bolus passes through the gastrointestinal tract.[25] Leptin acts together with GLP-1 and CCK in the brain, specifically in the nucleus of the solitary tract. However, this part of the brain also has neurons that are activated by PYY, independent of leptin.[2] Leptin also stimulates GLP-1 secretion in vivo and in vitro, through activation of LEPR, which can also contribute to satiety.[26] Furthermore, insulin exerts a satiety role in the hypothalamus that is similar to leptin´s action, stimulating ARC neurons, which express anorexigenic neuropeptides.[27] Leptin can impair insulin gene expression and glucose-stimulated insulin secretion, which can cause glucose levels to adapt to body fat stores, as well as affect appetite.[2,25]

a. Short-chain fatty acids (SCFA)

The interest in the microbiota has been growing, as this is the set of microorganisms that live in animal bodies and can interact with different organs and tissues.[28] Probiotics are live microorganisms that can be consumed in the diet with the capability to change the composition and activities of the microbiota – leading to benefits to the host.[29] Prebiotics are nutrients that can improve the quality of the microbiota. These nutrients are mainly fibers that are fermented by microorganisms, resulting in the release of metabolites, which include SCFA.[29]

Short-chain fatty acids are a subset of saturated aliphatic fatty acids with six or less molecules of carbon. They are the main colonic bacteria metabolites, produced by the fermentation of non-digestible carbohydrates. Due to lack of human enzymes to degrade dietary fibers, these types of carbohydrates maintain their structure through the upper gastrointestinal tract. Then, they are fermented in the large intestine by anaerobic caecal and colonic bacteria, resulting in production of SCFA, which are 95% acetate

(2C), propionate (3C) and butyrate (4C). The main sources of SCFA are plant cell-wall polysaccharides (mainly insoluble fibers), oligosaccharides and resistant starches (RS). Therefore, the total concentration of SCFA reflects the type and quantity of food intake.[31-33]

Despite the gut lumen being the major site of production, the concentration of SCFA falls rapidly along the length of the colon as a result of their uptake by colonocytes. According to den Bensten et al (2013) SCFA's concentration in the distal colon can reduce to more than half compared to the proximal colon concentration. After production in the gut lumen, 95% of SCFA is rapidly absorbed by colonocytes, and only 5% is secreted in the feces. [31-33]

a. <u>Acetate, propionate and butyrate: production pathways</u>

The most abundant gut SCFA is acetate, which corresponds to around 60% of the SCFA in the feces. The major part of acetate is the result of pyruvate fermentation by many types of enteric bacteria. Another source of acetate is fermentation of formate through acetogenic bacteria.[34]

Propionate, in contrast, is produced by few bacteria genera through three main pathways. The most prominent is present in *Firmicutes* and *Bacteriodetes*, which utilize succinate as substrate. Another pathway converts lactate through enzymatic reactions by the *Veillonellaceae* and *Lachnospiraceae* families. Finally, there is the conversion of deoxy-sugars, like fucose and rhamnose, to propionate, found in proteobacteria.[33,34]

Lastly, butyrate is synthetized via two pathways. Butyryl-CoA is converted to butyrate in a single enzymatic step by the major part of the intestinal microbiota, such as *Faecalibacterium*, *Eubacterium*, and *Roseburia*. There is also a pathway via butyrate kinase that is limited to *Coprococcus* species. The concomitant production of butyrate and propionate by the same bacteria is rare.[33,34]

b. Acetate, propionate and butyrate: substrates for production

Resistant starches (RS) are fermentable fibers and a type of prebiotic, as they provide energy for the microbiota. As they are not digested by the body, RS are fermented in the large intestine, resulting in production of SCFA and lowering of the gut pH.[35-37] Studies verified that higher consumption of RS can influence the types of bacteria found in the intestine. For example, some studies show that it can lead to lower levels of *Firmicutes* and higher levels of *Bacteriodetes*.[37] In addition, RS have been related to positive physiological effects, like satiety, controlled levels of blood lipids and increased insulin sensitivity.[35] There are four types of RS:

- RS1: entrapped in a nondigestible food matrix, is found in whole grains and pulses; [35,37]
- RS2: ungelatinized starch granules present in raw potatoes and cornstarch. The FDA allows Hi-maize 260 or high-amylose maize RS type 2 (HAMRS2) to be added and written on food labels, which is the type used as a supplement in research studies; [35,37]
- RS3: results from the degradation of cooked starches that become cold, such as a cooked potato that is refrigerated;[37]
- RS4: chemically modified starches, usually added to breads and cakes;[37]

Another SCFA substrate is inulin, a dietary fiber from the fructan carbohydrate subgroup, which contains chains with two to sixty monomers. Therefore, inulin can be classified as an oligo- or a polysaccharide that is almost insoluble in water. It is not digested by the gastrointestinal tract, which makes it possible to be fermented by the colonic microbiota, increasing SCFA formation. [38,39] This fiber is naturally present in onion, leek, garlic, banana, wheat, rye, barley, artichoke, chicory root, asparagus and dandelion root. It is used in the food industry to gelatinize, increase viscosity, improve organoleptic properties, sweeten, and increase fiber content.[38]

c. Acetate, propionate and butyrate: roles

Acetate, propionate and butyrate are important substrates for maintenance of gut integrity, because they take part in the integrity of the epithelial barrier through the regulation of tight junction proteins, therefore improving intestinal barrier function. These fatty acids also help in the control of gastrointestinal pH, colonic mobility and blood flow, which affect nutrient uptake and absorption. Besides, more than half of colonocytes' energy comes from butyrate, which induces proliferation of healthy cells.[32,33]

Moreover, SCFA have an effect on glucose and lipid metabolism trough AMPK. A homeostatic signal stimulated by propionate and butyrate in the hepatic portal system leads to gluconeogenesis. These SCFA are also inversely related to plasma insulin levels and insulin response, inducing improved glucose control. Regarding lipid homeostasis, propionate can stimulate fatty acid oxidation while inhibiting de novo lipogenesis and cholesterogenesis, which uses acetate as a precursor. Beyond that, SCFA are associated with increased fatty acid oxidation and decreased adipogenesis.[31,33]

Recent studies have verified that SCFA also participate in the regulation of the immune system, mainly due to their effect in controlling inflammatory responses and through the gut-liver axis. Butyrate can inhibit nuclear factor kappa- β activation in macrophages, and, like propionate, can inhibit histone deacetylation. Various studies have inversely related higher content of SCFA with less inflammation.[32,33]

d. Acetate, propionate and butyrate: action on Free Fatty Acids Receptors (FFARs)

Free Fatty Acids Receptors belong to the G protein-coupled receptor family, which contains 90 receptors that respond to a variety of ligands, such as purinergic nucleotides, lipids, leukotrienes, proteases and chemokines. Fatty acids are ligands for GPR40 to 43, which are also called Free Fatty Acids Receptor (FFAR). Two of those receptors are targeted by SCFA: FFAR2 (GPR43) and FFAR3 (GPR41). These

two receptors share around 40% of their peptide sequence, and more details about them can be seen at Table II.[32,40,41]

TABLE II

DETAILS ON FFAR2 AND FFAR3

	FFAR2	**FFAR3**
TISSUE OF EXPRESSION	Mainly immune cells, but also adipose tissue, enteroendocrine cells, distal ileum, colon and pancreas.[31,41]	Mainly adipose tissue, but also enteroendocrine cells, pancreas, spleen, lymph nodes, bone marrow and peripheral mononuclear cells, like monocytes. [31,41]
MAIN SCFA LIGAND	Acetate = propionate > butyrate.[31,32]	Propionate = butyrate > acetate.[31,32]

3. Potential association of SCFA and leptin metabolism

It has been verified that SCFA are the main ligands for FFAR2 and FFAR3, a type of receptors found in different tissues. Some studies have already related activation of FFAR2 and FFAR3 with satiety hormones´ synthesis and signaling, such as increase GLP-1 and PYY expression, causing decreased appetite and increased energy expenditure.[32,33,42-44] In addition, the activation of FFAR3 has been related to increased leptin release and expression *in vitro*.[45]

It is still unclear if SCFA are related to leptin metabolism via additional mechanisms, such as FFAR3, and the relationship of SCFA with dysbiosis. It also remains unclear if there is a dose-response for the possible effects of SCFA on leptin, as well as species-specific responses. The roles of SCFA in the body is still a relatively novel area of study, with no consensus about mechanisms of action and consequences. Hence, a comprehensive systematic review to integrate the existing information about leptin and SCFA is important.

4. Objective

This systematic review aims to assess the effects of SCFA in leptin´s synthesis and signaling. Secondary objectives, if this association exists, are to understand the mechanisms and consequences of this association, as well as to recognize possible clinical uses.

METHODS

According to Uman (2011), a systematic review "involves a detailed and comprehensive plan and search strategy derived *a priori*, with the goal of reducing bias by identifying, appraising, and synthetizing all relevant studies on a particular topic" trough eight stages. Therefore, this review was elaborated based on these eight stages:[46]

1. Formulate question

As explained on the rationale of this thesis, there is a lack of consolidated information regarding the association of SCFA with leptin metabolism. This led to formulation of the question "Do short-chain fatty acids affect leptin synthesis and signaling?" that is the guide to this systematic search.

2. Define inclusion and exclusion criteria

Based on the question above, the inclusion criteria were: (I) primary articles, (II) written in English, (III) which assessed leptin signaling or production as variable, (IV) SCFA were the intervention, a mechanism or an outcome to justify the results. On the contrary, (I) secondary articles, such as reviews and editorials were excluded, and (II) studies that were not related to SCFA were excluded.

3. Develop search strategy and do the search

Initially, a free search on PUBMED and Google Scholar regarding studies about leptin and SCFA was made. Among these studies, there were four selected as "must include". These four studies were used to define the key words and MeSH terms for the systematic search.

The final search was made in PubMed/Medline in March 2018 using the following keywords including MeSH terms or Text Word: leptin and any of these – propionate, butyrate, acetate, short-chain fatty acids, high-amylose maize resistant starch type 2 and resistant starch.

4. Select studies

The app Rayyan was used to select or exclude articles.[47] All studies were screened separately by two investigators (FG and GF) based on the title and abstract. Then, the remaining articles were screened by full-text reading. Thereafter, the articles were assessed for eligibility. Moreover, the reference lists from the included articles and the reviews were checked to search for other relevant studies.

5. Extract data

After carefully reading the articles, a table with the summary of all data was prepared with the following information: reference, sample (human, animal or in vitro), details about sample, details about methods, SCFA as an intervention/mechanism/outcome, statistical power of the study, results about leptin, other important results, association between SCFA and leptin.

6. Assess quality

Two validated quality assessment tools were used in this review, based on their objective characteristics to analyze each study.

- Human studies were analyzed using the checklist elaborated by the Academy of Nutrition and Dietetics.[48]
- Animal studies were assessed by the ten questions developed by the Systematic Review Center for Laboratory animal Experimentation (SYRCLE).[49] There are no validated tool to assess the quality of in vitro studies. Therefore, these studies were analyzed using the SYRCLE tool. This decision was based on a presentation by the National Toxicology Program from the Office of Health Assessment and Translation that mentioned that in vitro studies should be assessed by the same characteristics as animal studies.[50]

A table with the summary of this assessment was elaborated, with 10 criteria analyzed separately:

- Selection Bias (Randomization)
- Performance Bias (Humans: allocation concealment and baseline characteristics / Animals: controlled environment, similar gender, age and weight)
- Detection Bias (Blinded outcome assessment)
- Attrition bias (Reporting drop-outs and withdraws; Intention to treat analysis)
- False positive report bias (Adequate sample sizes - if not, report as a limitation)
- Statement of possible conflict of interest
- Statistical validity (accuracy of results based on data analysis)
- Construct validity (variables accurately measure what was intended to measure)
- External validity (results applicable to other people in different settings at a different time - generally applicable across biology)
- Internal validity (causal interpretation to the relationship found between the variables)

7. Summarize evidence and interpret results

The results table and the quality assessment form the core of the results. We also compared these data with information from relevant scientific literature, and conclusions about the association of the two main variables.

8. Disseminate findings

Beyond this Master´s thesis presentation to a Committee and availability on the University of Illinois at Chicago's (UIC) library, the authors intend to publish the results as a peer-reviewed article.

RESULTS AND DISCUSSION

Our systematic search found 573 articles. After screening titles and abstracts, 487 were excluded. The remaining 86 were read carefully: 35 were excluded and 51 assessed for eligibility. All of them had references reviewed, which contributed to four more articles to be included. After the assessment, 36 studies were included in this review (Figure 1 and Table III).

The characteristics and main outcomes of the 36 articles included in this systematic review are presented in APPENDIX 1. In summary, most studies were performed in animals (n = 24), followed by *in vitro* studies (n = 8) (one of which also performed part of the intervention *in vivo*) and then human studies (n = 4). The majority of the studies had one or more SCFA as an intervention (n = 16), while the rest were divided into studies that analyzed SCFA as a mechanism for modulation of leptin (n = 10) or as a dependent variable (n = 10). The studies were published between 2002 and 2018, with the majority published since 2014 (n = 21).

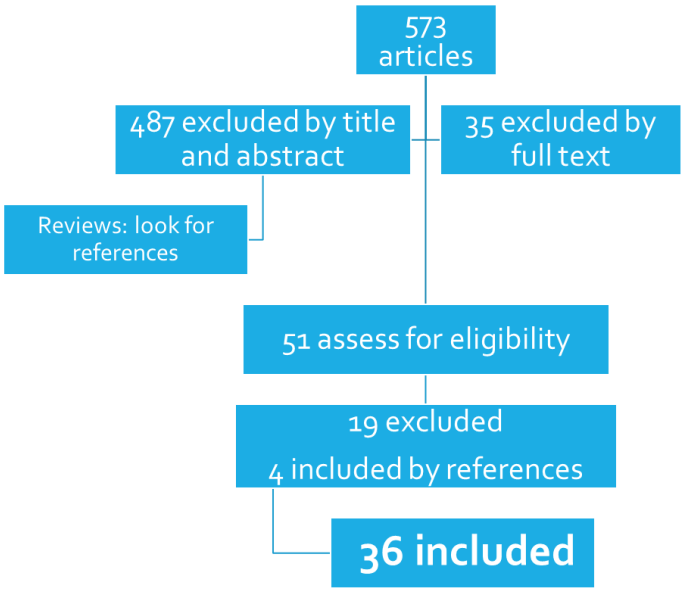

Figure 1. Study selection process

TABLE III

REFERENCES OF THE INCLUDED STUDIES

Authors	Year	Sample	Title
Adam CL, Gratz SW, Peinado DI, Thomson LM, Garden KE, Williams PA, Richardson AJ, Ross AW. (79)	2016	Animal	Effects of Dietary Fibre (Pectin) and/or Increased Protein (Casein or Pea) on Satiety, Body Weight, Adiposity and Caecal Fermentation in High Fat Diet-Induced Obese Rats
Al-Lahham SH, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, Rezaee F, Venema K, Vonk RJ. (56)	2010	In vitro (ex vivo)	Regulation of adipokine production in human adipose tissue by propionic acid.
Bradford BJ, Oba M, Ehrhardt RA, Boisclair YR, Allen MS. (86)	2006	Animal	Propionate is not an important regulator of plasma leptin concentration in dairy cattle.
Brøkner C, Austbø D, Næsset JA, Blache D, Bach Knudsen KE, Tauson AH. (88)	2016	Animal	Metabolic response to dietary fibre composition in horses.
Den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, Oosterveer MH, Jonker JW, Groen AK, Reijngoud DJ, Bakker BM. (63)	2015	Animal	Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation.
Fernández-Navarro T, Salazar N, Gutiérrez-Díaz I, de Los Reyes-Gavilán CG, Gueimonde M, González S. (92)	2017	Human	Different Intestinal Microbial Profile in Over-Weight and Obese Subjects Consuming a Diet with Low Content of Fiber and Antioxidants.
Frost G, Cai Z, Raven M, Otway DT, Mushtaq R, Johnston JD. (60)	2014	In vitro	Effect of short chain fatty acids on the expression of free fatty acid receptor 2 (Ffar2), Ffar3 and early-stage adipogenesis.
Fu C, Liu L, Li F. (87)	2018	Animal	Acetate alters the process of lipid metabolism in rabbits.
Hong J, Jia Y, Pan S, Jia L, Li H, Han Z, Cai D, Zhao R. (65)	2016	Animal	Butyrate alleviates high fat diet-induced obesity through activation of adiponectin-mediated pathway and stimulation of mitochondrial function in the skeletal muscle of mice.
Huazano-García A, López MG. (64)	2015	Animal	Agavins reverse the metabolic disorders in overweight mice through the increment of short chain fatty acids and hormones.
Jia Y, Hong J, Li H, Hu Y, Jia L, Cai D, Zhao R. (88)	2017	Animal	Butyrate stimulates adipose lipolysis and mitochondrial oxidative phosphorylation through histone hyperacetylation-associated β(3) -adrenergic receptor activation in high-fat diet-induced obese mice.
Lee SH, Hossner KL. (85)	2002	Animal	Coordinate regulation of ovine adipose tissue gene expression by propionate.
Li X, Xu Q, Jiang T, Fang S, Wang G, Zhao J, Zhang H, Chen W. (66)	2016	Animal	A comparative study of the antidiabetic effects exerted by live and dead multi-strain probiotics in the type 2 diabetes model of mice.

Li X, Wang E, Yin B, Fang D, Chen P, Wang G, Zhao J, Zhang H, Chen W. (80)	2017	Animal	Effects of Lactobacillus casei CCFM419 on insulin resistance and gut microbiota in type 2 diabetic mice.
Lin HV, Frassetto A, Kowalik EJ Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ. (62)	2012	Animal	Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms.
Marques TM, Wall R, O'Sullivan O, Fitzgerald GF, Shanahan F, Quigley EM, Cotter PD, Cryan JF, Dinan TG, Ross RP, Stanton C. (82)	2015	Animal	Dietary trans-10, cis-12-conjugated linoleic acid alters fatty acid metabolism and microbiota composition in mice.
Maziarz MP, Preisendanz S, Juma S, Imrhan V, Prasad C, Vijayagopal P. (95)	2017	Human	Resistant starch lowers postprandial glucose and leptin in overweight adults consuming a moderate-to-high-fat diet: a randomized-controlled trial.
Pelgrim CE, Franx BAA, Snabel J, Kleemann R, Arnoldussen IAC, Kiliaan AJ. (69)	2017	Animal	Butyrate Reduces HFD-Induced Adipocyte Hypertrophy and Metabolic Risk Factors in Obese LDLr-/-.Leiden Mice.
Priyadarshini M, Thomas A, Reisetter AC, Scholtens DM, Wolever TM, Josefson JL, Layden BT. (93)	2014	Human	Maternal short-chain fatty acids are associated with metabolic parameters in mothers and newborns.
Reid DT, Eller LK, Nettleton JE, Reimer RA. (89)	2016	Animal	Postnatal prebiotic fibre intake mitigates some detrimental metabolic outcomes of early overnutrition in rats.
Reygner J, Lichtenberger L, Elmhiri G, Dou S, Bahi-Jaber N, Rhazi L, Depeint F, Bach V, Khorsi-Cauet H, Abdennebi-Najar L. (84)	2016	Animal	Inulin Supplementation Lowered the Metabolic Defects of Prolonged Exposure to Chlorpyrifos from Gestation to Young Adult Stage in Offspring Rats.
Rivero-Gutiérrez B, Gámez-Belmonte R, Suárez MD, Lavín JL, Aransay AM, Olivares M, Martínez-Augustin O, Sánchez de Medina F, Zarzuelo A. (70)	2017	Animal	A symbiotic composed of Lactobacillus fermentum CECT5716 and FOS prevents the development of fatty acid liver and glycemic alterations in rats fed a high fructose diet associated with changes in the microbiota.
Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. (94)	2005	Human	Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism.
Si X, Shang W, Zhou Z, Shui G, Lam SM, Blanchard C, Strappe P. (71)	2018	Animal	Gamma-aminobutyric Acid Enriched Rice Bran Diet Attenuates Insulin Resistance and Balances Energy Expenditure via Modification of Gut Microbiota and Short-Chain Fatty Acids.
Soliman M, Kimura K, Ahmed M, Yamaji D, Matsushita Y, Okamatsu-Ogura Y, Makondo K, Saito M. (54)	2007	In vitro	Inverse regulation of leptin mRNA expression by short- and long-chain fatty acids in cultured bovine adipocytes.
Soliman MM, Ahmed MM, Salah-Eldin AE, Abdel-Aal AA. (55)	2011	In vitro	Butyrate regulates leptin expression through different signaling pathways in adipocytes.

Sugatani J, Osabe M, Wada T, Yamakawa K, Yamazaki Y, Takahashi T, Ikari A, Miwa M. (73)	2008	Animal	Comparison of enzymatically synthesized inulin, resistant maltodextrin and clofibrate effects on biomarkers of metabolic disease in rats fed a high-fat and high-sucrose (cafeteria) diet.
Thum C, McNabb WC, Young W, Cookson AL, Roy NC. (90)	2016	Animal	Prenatal caprine milk oligosaccharide consumption affects the development of mice offspring.
Toden S, Belobrajdic DP, Bird AR, Topping DL, Conlon MA. (81)	2010	Animal	Effects of dietary beef and chicken with and without high amylose maize starch on blood malondialdehyde, interleukins, IGF-I, insulin, leptin, MMP-2, and TIMP-2 concentrations in rats.
Wu W, Xie J, Zhang H. (91)	2016	Animal	Dietary fibers influence the intestinal SCFAs and plasma metabolites profiling in growing pigs.
Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M. (45)	2004	In vitro Animal	Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41.
Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. (72)	2013	Animal	Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion.
Yonekura S, Senoo T, Kobayashi Y, Yonezawa T, Katoh K, Obara Y. (58)	2003	In vitro	Effects of acetate and butyrate on the expression of leptin and short-form leptin receptor in bovine and rat anterior pituitary cells.
Yonekura S, Hirota S, Tokutake Y, Rose MT, Katoh K, Aso H. (59)	2014	In vitro	Dexamethasone and acetate modulate cytoplasmic leptin in bovine preadipocytes.
Yuan H, Wang W, Chen D, Zhu X, Meng L. (67)	2017	Animal	Effects of a treatment with Se-rich rice flour high in resistant starch on enteric dysbiosis and chronic inflammation in diabetic ICR mice.
Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJ, Smith DM, Arch JR. (57)	2010	In vitro	Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids.

Authors ( ): number of the reference in the cited literature.

A) Quality assessment

The results of the risk of bias assessment are presented below, based on standard criteria (51;52). Figure 2 shows that none of the studies showed risk of bias regarding construct validity and statistical validity (n = 36), and most of them did not present conflict of interest (n = 35) or performance bias (n = 30). On the other hand, the majority of studies presented a high risk of attrition bias (n = 35) and detection bias (n = 31), meaning that there was no mention of withdrawals or blinding procedures. Regarding

internal and external validity, as well as false positive bias, it is not possible to reach conclusions, as most studies were not performed in humans. Table IV presents the quality assessment for each included study.

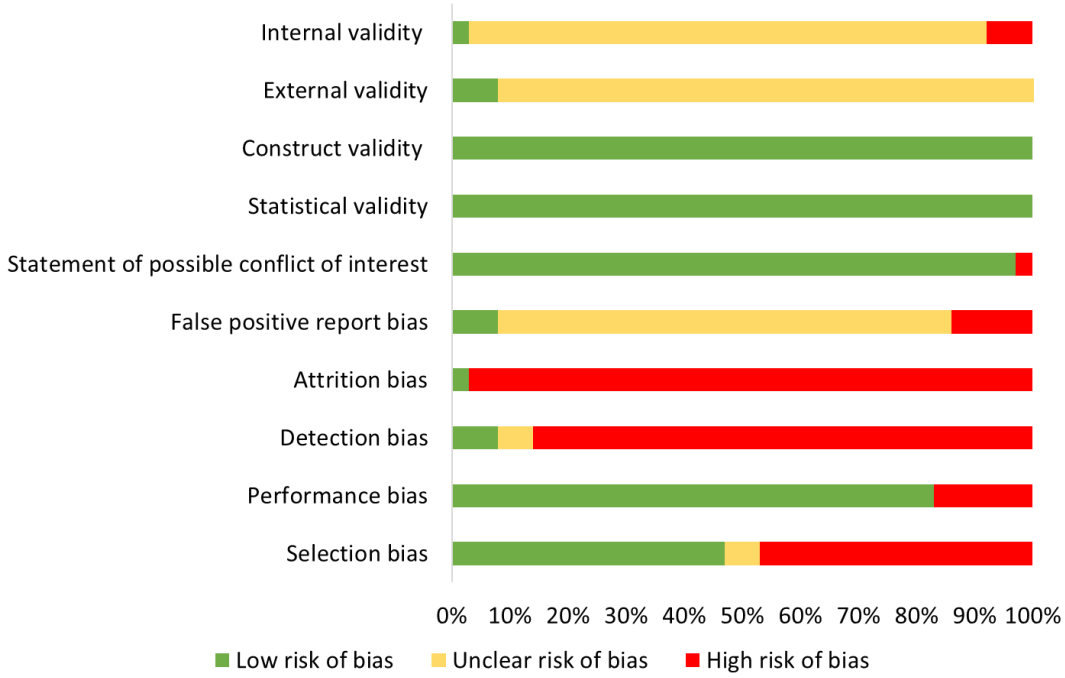

Figure 2. Risk of bias graph

TABLE IV

RISK OF BIAS OF EACH INCLUDED STUDY

Reference	Type of sample	Selection bias	Performance bias	Detection bias	Attrition bias	False positive report bias	Possible conflict of interest	Statistical validity	Construct validity	External validity	Internal validity
Adam CL, 2016	Animal					Not mentioned				N/A	N/A
Al-lahham SH, 2010	In vitro (ex vivo)					Not mentioned				N/A	N/A
Bradford BJ, 2006	Animal					Not mentioned				N/A	N/A
Brokner C, 2016	Animal									N/A	N/A
Den besten G, 2015	Animal									N/A	N/A
Fernandéz-Navarro T, 2017	Human										
Frost G, 2014	In vitro					Not mentioned				N/A	N/A
Fu C, 2017	Animal					Not mentioned				N/A	N/A
Hong J, 2016	Animal					Not mentioned				N/A	N/A
Huazano-Garcia A, 2015	Animal					Not mentioned				N/A	N/A
Jia Y, 2017	Animal					Not mentioned				N/A	N/A
Lee SHE, 2002	Animal									N/A	N/A
Li X, 2016	Animal	N/A		N/A		Not mentioned				N/A	N/A
Li X, 2017	Animal	N/A		N/A		Not mentioned				N/A	N/A
Lin HV, 2012	Animal					Not mentioned				N/A	N/A
Marques TM, 2015	Animal					Not mentioned				N/A	N/A
Maziarz MP, 2017	Human										
Pelgrim CE, 2017	Animal					Not mentioned				N/A	N/A
Priyadarshini M, 2014	Human										
Reid DT, 2016	Animal					Not mentioned				N/A	N/A
Reygner J, 2016	Animal					Not mentioned				N/A	N/A
Rivero-Gutiérrez B, 2017	Animal					Not mentioned				N/A	N/A
Roberston MD, 2005	Human										
Si X, 2018	Animal					Not mentioned				N/A	N/A
Soliman M, 2007	In vitro					Not mentioned				N/A	N/A
Soliman MM, 2011	In vitro					Not mentioned				N/A	N/A
Sugatani J, 2008	Animal									N/A	N/A
Thum C, 2016	Animal					Not mentioned				N/A	N/A
Toden S, 2010	Animal					Not mentioned				N/A	N/A
Wu W, 2016	Animal					Not mentioned				N/A	N/A
Xiong Y, 2004	In vitro / Animal					Not mentioned				N/A	N/A
Yadav H, 2013	Animal					Not mentioned				N/A	N/A
Yonekura S, 2003	In vitro					Not mentioned				N/A	N/A
Yonekura S, 2014	In vitro					Not mentioned				N/A	N/A
Yuan H, 2016	Animal					Not mentioned				N/A	N/A
Zaibi MS, 2010	In vitro					Not mentioned				N/A	N/A

Green: low risk of bias; yellow: unclear risk of bias; red: high risk of bias; N/A: not applicable for this methodology; Not mentioned: no mention about the impact of sample size in the results or discussion sections.

According to Guyatt et al. (2008), the quality of evidence "reflects the extent to which confidence in an estimate of the effect is adequate to support recommendations,"[53] which implies that in a systematic review the quality of the evidence reflects the range of confidence that the effect estimation is accurate. In this same paper, the authors mention factors that may decrease the quality of evidence, such as "study limitations, inconsistency of results, indirectness of evidence, imprecision and publication bias". In this systematic review, it was possible to critically analyze the studies limitations, such as the absence of blinding and dropouts (detection and attrition bias) in most of the studies. Only half of the studies

included in this review used some type of randomization (selection bias). On the other hand, all of the studies measured the variables accurately (construct validity); furthermore, there was accuracy of results based on statistical data analysis (statistical validity) and most of the studies performed have low risk of performance bias, trough allocation concealment (n = 29). Finally, the authors mentioned there was no possible conflict of interest in all but one study.

At the same time, there are factors that may increase the quality of evidence, such as large magnitude effects as well as plausible confounding and dose-response gradients. However, as most of the studies included in this review were *in vitro* or animal studies, assessing their quality was not straightforward, as only few validated tools are available for this purpose, meaning that it is hard to assess criteria like generalization and causal interpretation of studies (external and internal validity) in animal and *in vitro* studies. In addition, in these types of studies, sample size (false positive bias) is usually relatively small when compared to human randomized-control trials, aside from the fact that most studies do no report sample size calculations.[49]

B) Main findings

1. *In vitro* studies

In *vitro* studies used adipocytes from humans, cows, mice or rabbits incubated with one or more SCFA (n = 8). Most of them (n = 6) demonstrated that these fatty acids increase leptin expression in adipocytes.[45,54-57,59] One study found that butyrate can stimulate leptin expression in bovine pituitary cells but inhibits its expression in rat pituitary cells.[58] The remaining *in vitro* study found no statistically significant effect of acetate or propionate on leptin production, even though SCFA did activate FFAR2.[60]

The mechanisms by which SCFA increase leptin expression were studied *in vitro* in five studies. Adipocytes from cows, mice and humans respond to SCFA through FFAR2 and 3. These five studies used pertussis toxin (PTX), an inhibitor of FFAR on the cell membrane.[40] Four of these studies verified that PTX

treatment abolished propionate, butyrate and acetate stimulation of leptin expression, demonstrating that SCFA activate FFAR3 on adipocytes, increasing leptin expression. However, these studies could not conclude much about the role of FFAR2, due to the low expression of this receptor in adipocytes.[45,54-56] Zaibi et al (2010) also used PTX but concluded that propionate activates leptin secretion on adipocytes through both FFAR2 and 3. In another analysis, without PTX, this same group of authors verified that acetate did not stimulate leptin expression in cells from FFAR3 knockout mice. They discuss that this happened not only due to the absence of FFAR3, but also to the concomitant downregulation of FFAR2.[57] An additional pathway that might be related to increased leptin expression in response to SFCA is downregulation of protein kinase A (PKA).[55] These findings are in agreement with results that show that PKA inhibition leads to a significant rise in leptin secretion in rat adipocytes.[61]

The study that did not find significant effects of SCFA on leptin production hypothesized the result might be due to the use of preadipocytes instead of mature adipocytes.[60] However, two other studies included in this review also used adipocytes before cell differentiation and demonstrated induction of leptin by SCFA. However, the authors of these two studies used human[55] and bovine[59] preadipocytes whereas Frost et al (2014) used mouse preadipocytes. Therefore, the effect of SCFA on leptin production by preadipocytes may be species-specific.

Aside from the type of cell used, the concentration of SCFA used in the cell incubation is an important parameter to understand applicability *in vivo*. Most of the studies used around 1mM of SCFA to achieve the results, but some authors discussed that higher doses of SCFA, which are supra physiological, might lead to a different response in leptin expression. Actually, one study that incubated rat pituitary cells with 10mM of butyrate demonstrated a decrease in leptin expression. However, these are different cells types, which might also impact the result. [59] Similarly, authors that did not observe a significant impact of SCFA on leptin expression used higher doses than typically used in studies.[55,56,60] It is

challenging to determine an ideal dose for this type of study, because there is no evidence of the SCFA amount that reaches the adipose tissues after gut absorption.

To summarize, studies performed in vitro in adipocytes show that SCFA stimulate leptin expression through activation of FFAR3, which is highly expressed in adipose tissue. Despite FFAR2 being activated by SCFA, as verified in one study, it remains unclear if this receptor plays a role in leptin expression, as this receptor is expressed at low levels in adipocytes. One study verified that the inhibition of PKA by butyrate is probably related to the upregulation of leptin. Yet, these results should be analyzed with care as *in vitro* studies eliminate confounding factors, such as the influence of other hormones in leptin metabolism, as well as they may use concentrations of SCFA that do not correspond to the amounts present in the extracellular fluid of adipose tissue.[31]

2. Animal studies

a. Interventions aimed to modulate the effect of HFD in animals

Twelve studies used prebiotics and probiotics administered through the diet or by intravenous infusion to investigate the effects on modulation of HFD in mice or rats.[62-73] Overall, HFD led to increased body weight and leptin levels; the interventions were able to restore leptin levels to those observed in animals fed control diets.

Three studies determined that oral butyrate added to HFD led to less weight gain and, consequently, lower leptin levels compared to HFD alone.[65,68,69] The authors related the effect of butyrate to the activation of AMPK and PKA, in addition to decreased adipocyte hypertrophy and reduced activity of histone deacetylases. According to the literature, AMPK and histone deacetylases are related to lipolysis and adipocyte differentiation, respectively, which could be one of the causes to the suppressed weight gain.[74,75] Even though the interventions did not restore body weight to levels observed in animals fed control diets, leptin levels remained positively correlated with body weight and adiposity in mice.

Thus, butyrate was able to modulate HFD outcomes. None of these studies measured food intake, which would be an important variable to understand the mechanism involved in butyrate's modulation of adiposity and leptin levels.[65,68,69]

Similar results were found in two studies that verified that each of the SCFA, when orally consumed with a HFD, suppressed weight gain and leptin concentrations. Importantly, in one of the studies SCFA only had a minor effect on leptin expression in FFAR3-knockout mice, which again shows the importance of this type of receptor in mediating the effect of SCFA.[62] Once more, the activation of AMPK occurred in parallel to increased leptin, together with downregulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) caused mainly by propionate and butyrate.[63] These two substances take part on lipolysis, which could be associated with the weight control observed in animals receiving SCFA as compared to HFD alone.[74,75] Even though all doses of SCFA affected leptin expression, only propionate and butyrate affected food intake, which was decreased. It should be remembered that satiety and appetite are regulated through a complex system of many hormones, not only leptin.[24]

Another influence that these interventions have on leptin concentration is through improvement in dysbiosis, by modulating the quality of the microbiota and increasing individual SCFA content. Yuan et al (2017) found more caecum SCFA in diabetic mice that received oral RS. This increase in SCFA was concomitant with decreased serum leptin concentrations.[67] Likewise, Li et al (2016) used probiotics to restore leptin levels that were increased with HFD. Concomitant to that, there was a significant increase in fecal acetate and butyrate. However, there was no measurement of body weight or body composition in this study, which could have explained the observed reduction in leptin levels.[66] Yadav et al (2013) also verified that leptin levels in mice fed with HFD supplemented with probiotics were restored to those observed in mice fed a control diet. This occurred together with an increase in fecal butyrate, which might be related to improved microbiota. This last study measured body weight and fat mass, which were positively associated with leptin levels.[72]

An interesting result found in two studies previously mentioned was that butyrate and RS supplementation led to inverse results for leptin and FFARs levels.[67,68] As expected, the interventions caused increased FFAR2 gene expression and FFAR2 and 3 activation in adipose tissue, in response to increased SCFA. However, there was a decrease in serum leptin concentrations in animals supplemented with butyrate and RS compared to animals only receiving HFD, which is the opposite result compared to *in vitro* studies. Even though Jia et al (2017) only measured FFAR2 activation, and not FFAR3, their findings allow the assumption that leptin expression is more dependent of adiposity then on the effect of SCFA on FFARs, because butyrate caused loss of both weight and fat. However, Yuan et al (2016) focused on RS's ability to improve dysbiosis and decrease inflammation; thus, they did not measure body weight. However, based on other studies, the authors briefly discussed the possibility that the decrease in adiposity is the main cause for leptin´s decrease. These two studies demonstrate that SCFA absorbed in the intestine can reach the adipose tissue to activate FFARs *in vivo.* However, regulation of leptin production appears to be mainly related to adiposity rather than the presence of SCFA.

Huazano-Garcia & López (2015) verified that the prebiotics agavin from agave or inulin from chicory added to a HFD led to suppressed weight gain, increased colonic SCFA and restored leptin to control levels. These groups also showed decreased food intake, but this is possibly related to hormones other than leptin, as there was a decrease in ghrelin and an increase in GLP1 and insulin levels.[64] Inulin was another intervention associated with increased propionate and decreased leptin levels compared to a HFD, as well as decreased white adipose tissue. The authors did not discuss the mechanisms for their findings, but inulin has been associated with improved microbiota quality and, consequently, increased SCFA production.[73] Other studies, not included in this review, observed that inulin increases propionate and butyrate *in vitro* and in humans.[77,78] Another result that can be justified by the quality of the microbiota is the one by Rivero-Gutiérrez et al (2017). This group investigated the effect of a combination of probiotic and prebiotic, called symbiotic, added to HFD. This addition was able to decrease leptin

concentrations compared to the HFD group, even though there was no difference in body weight among groups. The results showed that the supplement led to fecal SCFA content similar to that observed in animals fed a control diet, which – surprisingly - was a decrease in SCFA contents. Even though this is the opposite of what was observed in most studies, the authors suggested this decrease was positive because it was an adaptation from the previous dysbiosis.[70] Hence, prebiotics and symbiotic were able to modulate HFD effects.

One study supplemented a HFD with rice bran, with or without addition of Gamma-Amino Butyric acid, which increased butyrate and propionate, which the authors mention could be the cause for the increased leptin levels compared to control. Moreover, there was a decrease in SOCS3 and AMPK expression in the hypothalamus, as well as an increase in LEPRb expression. These results could also contribute to the positive results possibly associated to the higher leptin levels and signaling, such as suppressed appetite and increased energy expenditure. However, this was the only study in which decreased adiposity and PPAR-γ were not proportionally associated with leptin concentrations. This was the only group of authors who mentioned increased leptin levels as positive, as it was associated with decreased food intake in obese rats.[71] As the results showed a decrease in food intake, these rats are probably leptin sensible.[1]

There were two studies that did not relate SCFA and leptin levels, as both variables were measured separately. The prebiotic pectin was added to a HFD control diet, as well as a HFD supplemented with casein or pea protein. In all cases, pectin was able to decrease leptin levels, which was attributed to the decreased body weight and fat mass compared to the groups without pectin. Although the authors did not mention the possibility of an association to leptin, pectin caused an increase in acetate and propionate, together with a decrease in butyrate.[79] Equally, a group of mice fed a HFD and probiotic had a slightly decrease in leptin levels compared to HFD alone, even without significant weight loss. Despite

the authors verified an increase in fecal acetate and butyrate, they associated the decrease in leptin to insulin levels.[80]

Overall, all the interventions studied in this group of reports caused a decreased in body weight and, consequently in adiposity, which seems to be the most relevant factor that affects leptin production. Moreover, SCFA production by the bacteria or as an intervention seems to be related to adequate leptin levels through different pathways, such as AMPK, PKA and PPAR-γ. The improvement in the quality of the microbiota can also play a role in this association.

b. Other interventions

The use of dietary supplements to improve the microbiota had positive impacts in controlling leptin levels in studies that used diets different from HFD. Toden et al (2010) investigated the outcome of RS added to diets that were similar in fat content but different in the types and amounts of protein. The use of RS was related to less plasmatic leptin and more portal and fecal SCFA regardless of the dietary protein. Their statistical tests showed that butyrate was inversely correlated with leptin levels ($r = -0.35$; $p < 0.05$), but acetate and propionate were not statistically significantly correlated with leptin.[81] In addition, Marques et al (2015) used Conjugated Linoleic Acid (CLA) in mice, which caused lower leptin concentration. As this supplement did not cause significant differences in body weight, the authors suggest that CLA altered the microbiota. This change caused an increased production of SCFA, which was probably the responsible for the decrease in leptin. The authors mention that SCFA can activate FFAR2 and 3, but they did not measure such activation.[82] Other studies, not included in this review, also found that CLA could alter the microbiota.[83]

Reygner et al (2016) used inulin as a supplementation in mice fed with chow diet and different amounts of an insecticide (Chlorpyrifos). Inulin was able to decrease leptin levels and significantly increase butyrate and total SCFA compared to control, which might be related to activation of FFARs, which the

authors did not measure.[84] A jugular infusion or an acute oral dose of propionate led to increased leptin mRNA in sheep and mice, respectively.[45,85] This was probably related to adipocyte differentiation, evidenced by an increase in PPARγ. However, there was no measure of body weight or food intake to understand the real cause of this rise in leptin. In addition, one study verified that physiologic jugular doses of propionate play a minor role in regulating plasma leptin in lactating dairy cows (increase of 8% without a linear response). The acetate dose used was not physiological, which might explain the lack of statistical significance between this SCFA and leptin.[86] Fu et al (2017) found that subcutaneous acetate injection in rabbits did not significantly affect leptin concentrations even though it increased AMPK, FFAR2 and 3, as well as PPAR-γ, which have all been related to leptin expression in other studies.[87] This might be another species-specific response.

The remaining animal studies measured leptin and SCFA, but the interventions did not significantly impact leptin production. The probable causes for lack of significance were small sample size[88], the different response for supplements in newborn rats[89], the justification that leptin results were only related to adiposity[90] and a similar weight loss in all groups[91]. Besides, their analysis did not reach any conclusion about the association of leptin and SCFA.

To sum up, in vivo animal studies consider the complex interactions of leptin and SCFA in the body, as the results reflect the pathways that might interact. Overall, aside the effects on modulation of adiposity, SCFA and interventions that stabilize the microbiota play an important role in controlling leptin levels.

3. Human studies

The systematic search led to the inclusion of four studies performed in humans. There were two observational studies, one crossover study and one randomized control trial. The samples ranged from 10 to 68 adults. One of the studies analyzed normal weight and overweight pregnant women, two studies

included only overweight and obese adults, while the other study had no pattern of weight (normal weight to obese individuals were included).[92-95]

One of the observational studies reached the conclusion that that higher BMI was related to higher leptin and fecal SCFA concentrations.[92] The authors found a statistically significant association that matches the *in vitro* studies cited above, where acetate was able to increase leptin expression. This was shown by the increased levels of fecal acetate and higher serum leptin in obese individuals, which are probably related to higher adiposity and inflammation, as determined by the high levels of Malondialdehyde, C-reactive protein and dysbiosis. Regarding acetate, a recent review affirmed that this is the SCFA more associated with obesity, because it is a substrate for cholesterol synthesis and lipogenesis in hepatocytes and other cells, such as adipocytes.[96] Another review pointed out that fecal SCFA might not be the best way to compare SCFA content between obese and non-obese subjects, due to the fact that this fecal concentration may be 20% higher in obese individuals as a compensatory mechanism to avoid SCFA obesogenic actions.[97]

Priyadarshini et al (2014) verified that obese pregnant women had significantly higher serum leptin than normal weight pregnant women. However, there was no statistically significant correlation between BMI and serum SCFA. On the other hand, the authors verified a negative correlation between serum propionate and maternal leptin. According to the authors, this could have a beneficial effect for the mother during pregnancy and probably to the newborn, as this SCFA was also negatively correlated with the newborn length and weight. Thus, greater serum propionate is associated with lower maternal leptin and could avoid large birth weights.[93] This study had a small sample size and did not investigate causality. Furthermore, the authors mentioned that serum SCFA probably represent the sum of fatty acids produced not only in the intestine but also in other organs, like the liver.[98]

The other two human studies compared the use of placebo or 30 grams of RS in adults.[94,95] One of them used a crossover design and found that RS had no statistically significant impact on leptin

concentrations compared to placebo despite increasing peripheral serum acetate and propionate. However, it should be highlighted that there was also no statistical significance among RS and body weight or fat mass. This study has limitations, including a small sample size (n = 10) and no standard BMI for the participants.[94] The other one was a randomized-control trial that found that subjects from the intervention group (RS) had decreased leptin levels compared to baseline. However, there was no statistical significance between intervention and control groups. Fatty acid oxidation, body weight and adiposity were not measured, but the authors attributed the decrease in leptin levels to these factors. There was no measurement of SCFA yet the authors suggest that some of the results may be due to the positive effect that RS has on SCFA production, [95] which has been seen in previous studies (99;100).[99,100] The absence of all these measures weakens the results of this study, as well as the fact that the baseline characteristics among groups were not statistically significant.

These four studies performed in humans had different samples and methods, making it difficult to discern a pattern of response. However, similar to animal studies, these studies show that leptin is mainly correlated with adiposity, though the microbiota may also participate in regulating leptin.

C) <u>Overall applicability of the evidence</u>

This systematic review shows that when SCFA are in contact with adipocytes, such as during *in vitro* studies, they activate FFAR3, which increases leptin expression.[101] However, *in vivo*, leptin concentration is correlated with many factors, with adiposity being dominant. Therefore, dietary factors that influence body weight and composition could be helpful to control leptin levels. The studies reviewed here showed that HFD stimulates weight gain and leptin expression and that some interventions related to SCFA were able to control these effects. The interventions were butyrate, probiotics and prebiotics, which all reduced weight gain and, consequently, decreased leptin levels. These interventions were able

to improve the overall quality of the microbiota, which is related to reduced inflammation and overall better health.[102]

In clinical practice, oral supplementation with butyrate or stimulation of SCFA production by prebiotics and probiotics can be a viable intervention for people that consume a HFD on a regular basis. The use of RS, a prebiotic, seems to be the best option for people that want to modulate the consequences of a HFD. Consumption of up to 25 g/d of RS is well tolerated, does not cause gastrointestinal symptoms, such as diarrhea and is associated with an increase in butyrate, which is the SCFA more related to positive outcomes.[103] The use of other prebiotics and probiotics also has positive outcomes, but their association with SCFA has been less studied.

D) Limitations

This systematic review has several limitations. Initially, it is only characterized by a systematic search, thus it is not a meta-analysis. Therefore, there was no statistical analysis of the data, which made the analysis of results more subjective. The main issue regarding this systematic review is the inclusion of animal and, specially, *in vitro* studies. These types of studies are not usually included in systematic reviews due to the lack of validated tools to objectively analyze them. However, considering the lack of available evidence in human subjects on the topic, it was essential to include *in vitro* and animal studies. Probably because of the wide inclusion criteria, the presence of studies with diverse methodologies can also be considered a limitation, as this makes it harder to compare conclusions among studies. Another limitation is that the review was conducted by an individual researcher, though there was validation from another researcher at the end of each step. Finally, we only included published papers in English, which can lead to language bias.

E) Future studies

The studies included in this review did not allow an exact conclusion regarding the relation between SCFA and leptin. Therefore, the details about the association between these variables still need to be clearly established. Future randomized control trials in humans should use SCFA as an intervention, perhaps adding SCFA to a regular diet or using fortified products, such as yogurts or breads. Similarly, control trials with inulin or RS, measuring SCFA and leptin as an outcome, would be important to understand the causality of the association. It would also be important to have more studies with pregnant women, as this appears to be a population that could benefit from more knowledge about the microbiota and leptin.

FINAL CONSIDERATIONS

The findings of the current systematic review allow us to conclude that the association between SCFA and leptin is not completely clear. It is possible to affirm that SCFA can affect leptin synthesis, mainly through the activation of FFAR3 in adipocytes. It seems that FFAR2 has a minor role in leptin´s synthesis, because it is expressed more in immune cells than in adipocytes. There is no evidence that SCFA play any role in leptin signaling, which was one of the original hypotheses considered by the author in the beginning of the review.

It has been shown that SCFA reflect the microbiota´s quality, which means that interventions that improve dysbiosis and balance SCFA production can have positive outcomes, such as adequate leptin levels. Besides, more *in vivo* studies should be performed because *in vitro* studies do not reflect the complex interactions observed *in vivo*. Leptin expression depends on many factors, but mainly body fat, which is predominant compared to the effect of FFAR3 activation by SCFA.

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APPENDIX A

SUMMARY TABLE OF INCLUDED STUDIES

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Adam CL, 2016	Animal	48 obese male Rats	Other dependent variable	HFD + cellulose (control), HFD + pectin, control + casein, control +pea protein, HFD + pectin + casein, HFD + pea protein + pectin	ANOVA; Fisher; Pearson´s; P<0.05	Pectin: decreased plasma leptin in the three groups; Leptin correlated positively with body fat mass and intake, but no influence on appetite.	Pectin: lower body weight and less fat mass gain, daily food intake, more PYY and GLP1; increased acetate and decreased butyrate No effect of casein in SCFA; Pea protein: more butyrate, acetate and propionate.	No comments. Leptin was not correlated with SCFA as other hormones
Al-Lahham SH, 2010	In vitro (ex vivo)	Human subcutaneous adipocytes from females	Intervention	Different concentrations of propionate (0 to 10mM)	Student´s t test; Pearson´s; p<0.05	1 and 3mM: stimulated Leptin mRNA expression; 10 mM: no influence it; NSS correlation of leptin mRNA and other variables (body weight, etc); NSS higher dose effect on leptin x dose produced by the intestine.	Propionate + PTX (TNF inhibitor): no induction of leptin expression.	Propionate stimulate leptin expression in different adipose tissues due to activation of FFAR3.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Bradford BJ, 2006	Animal	32 multiparous lactating cows and 12 lactating cows	Intervention	1) Chow diet, later added 1040umol/kg propionate on jugular vein 2) 0, 260, 520, 780, 1040 and 1300 mmol of propionate; constant 1300 mmol of propionate with acetate	Turkey´s, Cook´s D	1) Decreased plasma leptin after infusion (100min); pre infusion leptin was related with body condition; 2) NSS differences on leptin concentration (with acetate or propionate); Dose very different from those produced by intestine.	Based on experiment 2: acetate decrease with propionate.	Propionate: minor role in leptin´s regulation in lactating cows - only short-term effect. Leptin before infusion is psitevly correlated with body condition
Brokner C, 2016	Animal	Four horses	Other dependent variable	Treadmill + 4 types of diets with different content of fibre and starches	Pearson; P<0.05	M (medium fibre): higher leptin level; BB (more starch): lowest leptin concentration; A lot of NSS results;	Acetate > propionate > butyrate (in order of production) - mostly on BB (more starch and less fibre). Diet affected fecal propionate and butyrate.	No comments

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
den Besten G, 2015	Animal	Male mice	Intervention	Control, HFD, HFD with one of the SCFA	ANOVA, Turkey test; $p<0.05$	SCFA: leptin similar to control levels (decreased compared to HFD)	SCFA: More energy expenditure and lipid oxidation, reduced body weight gain; SCFA: less PPARg and more UCP2 and AMPK in some cases (some were NSS)	SCFA activate leptin expression, also less adipose tissue is related to less leptin levels
Fernandéz-Navarro T, 2017	Human	68 adults (80% female, eutrofic, overweight and obese)	Mechanism	Cross-sectional: FFQ, anthropometrics, blood analysis,fecal SCFA	Kolmogorov-Smirnov test; chi-squared, t-test; $p<0.05$	Obese: more leptin; NSS in leptin concentrations of clusters (eutrofic x obese).	Obese: higher metabolic rate, percentage of fat and SCFA; Cluster 2: more acetate; NSS for propionate and butyrate.	More obesity leads to more SCFA, mainly acetate which are related to increased levels of leptin.
Frost G, 2014	In vitro	Preadipocytes (from 3T3-L1 cells that are derived from mouse 3T3 cells that is used in biological research on adipose tissue)	Intervention	2 experiments: exposition to SCFA (different timing with acetate at 1um) or 1um propionate x control	ANOVA and Bonferoni´s	Time of exposure increased leptin concentration. 10x more concentration of SCFA: no effect on FFAR2 mRNA or leptin.	Low concentrations: do not affect FFAR.	NSS association.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Fu C, 2017	Animal	8 rabbits	Intervention	Subcutaneous acetate injection	Bartlett´s test, ANOVA; p<0.05	NSS on plasma leptin levels, upregulation of LEPR.	Upregulation of FFAR, PPARgama and cAMP.	Increased AMPK can be related to leptin as it affected LEPR
Hong J, 2016	Animal	36 male mice	Intervention	Control and HFD (become DIO mice), later HFD control and HFD + buyrate	ANOVA; Bonferroni; p<0.05	Butyrate: leptin similar to control levels (decreased compared to HFD) --> response to body fat and fat No changes in leptin receptor mRNA.	Butyrate: increased UCP2, NSS difference regarding FFAR2 and 3	Mainly related to adiposity
Huazano-Garcia A, 2015	Animal	32 male mice	Mechanism	Control and HFD - later: HFD in 3 groups control, agavin or chicory inulin	Student´s t test; ANOVA; p<0.05	Agavin and inulin group: lowest leptin levels (similar to standard diet); Even though GLP1 and insulin did not have SS results, leptin was similar to standard diet.	Agavin and inulin: 9% of weight loss, and less energy intake; less intestinal pH probably Agavin: more SCFA compared to HFD in three segment of intestine	PCA plot gathers all the results as factors for butyrate positive effects
Jia Y, 2017	Animal	Healthy mice	Intervention	Control diet and HFD (45% - became obese mice)), later HFD + butyrate	Western blot; ANOVA; p<0.05	Butyrate: leptin similar to control levels (decreased compared to HFD);	Butyrate: reduced body weight, decreased fat deposits size and adipocytes; induced FFAR2 signaling (opposite to leptin).	No comments about mechanisms.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Lee SHE, 2002	Animal	Four rams	Intervention	Jugular infusion of saline or 64umol/min/kg of propionate	Split-plot; $p<0.05$	Increased leptin mRNA.	Propionate: reduced mRNA of UCP2 and increased PPARg No measure of body weight and food intake.	Propionate: +45% more than control - probably leading to reduced feed intake and enhanced energy expenditure.
Li X, 2016	Animal	40 male mice	Other dependent variable	5 groups: control, HFD, HFD + antidiabetic, HFD + live probiotic, HFD + dead probiotic (5 strains 8 x 10 a 9)	Turkey´s and ANOVA; $p<0.05$	Probiotic groups: decrease in leptin levels (compared to HFD diabetic).	HFD diabetic: less acetate and butyrate, probiotic increased acetate and live probiotic increased butyrate; No measure of body weight.	No comments - imply the changes in metabolic parameters are due to SCFA and less inflammation, but not specific about leptin
Li X, 2017	Animal	32 male mice	Other dependent variable	Control diet and HFD in 3 groups: diabetic control, antidiabetic group and probiotic group ($8 \times 10^{1°}$ L casei)	Tukey´s test; $p<0.05$	DC: elevation of leptin compared to control; probiotic lowered it in diabetic mice, probably due to less insulin resistance and increase in insulin secretion.	Probiotic: NSS on body weight, improved inflammation, affected significantly butyrate with a small increase compared to control, acetate and total SCFA increased but not statistically significant.	No comments - states that probiotic could help on leptin resistance cases due to insulin

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Lin HV, 2012	Animal	Male mice	Intervention	Control and HFD, with latter adition of all three SCFA; latter FFAR3-/- mice	Student´s t test; ANOVA; p<0.05	Butyrate: fasting leptin similar to control levels (decreased compared to HFD); FFAR3 -/- showed normal leptin levels, compared to control.	Butyrate and propionate: blocked HFD weight gain (probably related to supress food intake), acetate only 40% less (increased metabolic rate or reduce absorptive efficiency). FFAR3-/-: NSS in body weight compared to normal mice, but 9% increased food intake	Leptin was less affected in FFAR3 knockouts, but this hormones was nos aded to the analysis among SCFA and hormones
Marques TM, 2015	Animal	16 male mice	Mechanism	Control or Conjugated Linoleic Acid (CLA)	Student t test; Kruskal-Wallis; Mann-Whitney; p <0.05	CLA: lower leptin concentration - proportional to amount of fat.	CLA: decrease in body fat; increase in all three SCFA (+34% compared to control) probably related to the difference in bacteria.	Adposity and activation of FFAR3 mght be the reponsible

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Maziarz MP, 2017	Human	25 healthy overweight adults	Other dependent variable	RCT, double-blind: 30g RS or control (muffins)	Mann Whitney test; Wilcoxon signed-ranked test; Pearson´s; p<0.05	RS: only group with difference of leptin AUC from baseline to the end of study (decrease), as well as decrease in postprandial --> from baseline to week 6; Total body fat correlated with AUC leptin; NSS among groups at week 6 (control and RS), but baseline were very different.	RS: did not change body composition; enhanced fat oxidation; No measure of SCFA.	As both groups had similar fat mass, could be one reason for them not to differ. SCFA poduced in the intestine might be one of the mechanisms responsible for the effect in leptin
Pelgrim CE, 2017	Animal	60 mice with sensivity to develop HFD obesity	Intervention	Control, HFD (became obese mice), HFD + butyrate	ANOVA, Pearson´s, p<0.05	Butyrate: leptin similar to control levels (decreased compared to HFD); Leptin was positively correlated with body weight adipocyte size and inflammation.	HFD: increased adipocyte size, but butyrate reduced it, as well as decreased body weight and adiposity.	Butyrate was able to attenuate HFD effects on leptin, no comments about mechanisms.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Priyadarshini M, 2014	Human	10 obese pregnants and 10 normal weight pregnants	Mechanism	No intervention - observational	T test; p<0.05	Obese: 2x more leptin concentration.	Acetate greater than propionate, followed by butyrate.	Serum propionate correlated negatively with maternal leptin - beneficial effect for the mother during pregnancy and probably to the newborn; Acetate and butyrate NSS with leptin.
Reid DT, 2016	Animal	12 litter rats	Other dependent variable	Control or prebiotic fibre (oligofructose OFS)	ANOVA; Tukey´s; P<0.05	NSS leptin, but area under the secretory curve tended to be lower in OFS.	OFS: lower body weight and energy intake; more expression of FFAR - probably due to upregulation by SCFA.	No comments
Reygner J, 2016	Animal	32 Pregnant rats and 8 male pups	Other dependent variable	6 groups: control, CPF0, CPF 1, CPF3.5, inu0 and inu1 (CPF: chlorpyrifos inseticide / Inu: chicory inulin)	Mann Whitney test; p<0.05	Inulin: decreased leptin levels.	Neither treatments affected weight gain, food or drink intake; Inulin increased SCFA production, with significant increase of butyrate and total.	SCFA may explain reduction of insulin levels due to FFAR2 in the adipose tissue - not specific about leptin.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Rivero-Gutiérrez B, 2017	Animal	Male rats	Mechanism	Control, HFD, HFD + symbiotic (Fructo-Oligosaccharides + bacteria)	ANOVA; $p<0.05$	Symbioitic group: decrease leptin level (compared to HFD).	HFD: decreased colonic FFAR3, increased SCFA; HFD + symbiotic: more colonic FFAR3 compared to HFD; less SCFA compared to HFD.	SCFA and leptin are positively proportional, but FFAR3 is the opposite
Roberston MD, 2005	Human	10 healthy adults (BMI from 18.4 to 32.3)	Mechanism	Single blinded, crossover: 30g of oral RS or placebo in usual diet	Student´s t test, Pearson´s; $p<0.05$	RS: NSS on leptin concentrations.	RS: systemic concentrations of acetate and propionate were higher, NSS for butyrate.	No mechanisms discussed.
Si X, 2018	Animal	24 obese male rats	Mechanism	HFD, HFD + rice bran or HFD + rice bran + Gamma-Amino Butyric acid (GABA functional non protein aa)	Kruskal-Wallis; $P<0.05$	GABA:increased serum leptin and leptin receptor expression.	GABA: lower body weight gain and fat weight compared to control; less food intake, SOCS3 and AMPK, less acetate and more propionate and butyrate - probably due to changes in microbiota.	Increased butyrate and propionate can be related to increased serum leptin in GABA groups. Only study that saw increase as positive, as controlled food intake, because rats responded to leptin

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Soliman M, 2007	In vitro	Subcutaneous adipocytes was obtained from 3 non-pregnant and non-lactating cows	Intervention	Absence or presence of acetate, butyrate, propionate and other fatty acids	Northern blot, Western blot, ANOVA and Fischer´s. P <0.05	Increased leptin expression.	PTX treatment reduced basal and acetate-induced leptin expression; MAPK inhibitor did not inhibit acetate effect on leptin; Acetate and insulin had similar effects on leptin expression.	Any of the SCFA enhanced leptin expression - higher dose had the higher expression (1mM significant). Probably due to FFAR and similar to mice cells. These responses might be different in different cells with these receptors: mammary epithelial, pituitary and adipocytes.
Soliman MM, 2011	In vitro	Human preadipocytes	Intervention	Incubated with or without butyrate at 0.5, 1 or 5 mM	Western blot, Northern blot, ANOVA with p<0.05	Butyrate induced leptin expression at lower doses but the higher dose (5mM) inhibited leptin mRNA expression;	Similar response to bovine and mice adipocytes, as well as FFAR3 transfected cells.	Butyrate and leptin are probably related by FFAR3 (use of PTX) and phosphorylation of MAPK and PKA

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Sugatani J, 2008	Animal	Male Wistar rats	Other dependent variable	Standard diet, 5% inulin or resistant maltodextrin-supplemented standard diet, high fat and high sucrose diet (cafeteria), or 5% inulin or resistant maltodextrin-supplemented high fat high sucrose diet	ANOVA; $p<0.05$	Cafeteria diet: elevated serum leptin levels --> inulin and resistant starch decreased these levels (specially RS); Standard diet: NSS.	Cafeteria + inulin: less food intake. Cafeteria diet: less portal plasma propionate and butyrate. Due to high fructose content can lead to hyperleptinemia, as well as high fat content; Inulin and RS: increased portal plasma propionate in both diets.	No comments.
Thum C, 2016	Animal	21 Male and 42 female mice	Other dependent variable	Control diet, Galacto-oligosaccharides (GOS; more protein) or caprine milk oligosacharides (CMO)	ANOVA; $p<0.05$	No effect on serum leptin concentration; After 30 days of weaning CMO diet (from the mothers) lead to higher concentration than control in pups;	CMO diet increased visceral fat; GOS higher concentration of propionate; GOS and CMO lead to less butyrate;	Results related to adiposity.
Toden S, 2010	Animal	96 adult male rats	Mechanism	12 groups: 15, 25 or 25% beef, 13, 22 or 30% chicken with or without RS (similar fat content)	ANOVA; Pearson´s; $p<0.05$	RS: less serum leptin concentration, independent on type and level of protein.	RS: more hepatic SCFA, higher values in beef groups	Serum leptin correlated negatively with portal vein plasma butyrate, but NSS with acetate and propionate.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Wu W, 2016	Animal	18 male pigs	Mechanism	3 groups: inulin, celulose (MCC) and corn starch	ANOVA, p>0.05	Fasting leptin did not differ among groups (NSS).	Similar feed intake; Inulin: greater body weight than MCC; greater propionate and reduced acetate.	Even with the altered concentrations of SCFA, did not impact leptin levels as it was seen in other studies; But all groups had weight gain.
Xiong Y, 2004	In vitro Animal	Epididymal fat and adipocytes from mice Male mice	Intervention	Incubation with SCFA 2.5M propionate or placebo	ANOVA and Dunnet´s test	SCFA increased the level of leptin secretion; Propionate + insulin: can be coadministred to stimulate leptin production; Plasma leptin levels are elevated after propionate administration.	Acetic acids activates human FFAR3.	SCFA stimulate leptin production in adipocytes and whole animals; Supression of FFAR3 inhibit propionate effect; Propionate x food intake: no difference; FFAR2 role is not yet verified.
Yadav H, 2013	Animal	28 Male mice	Other dependent variable	4 groups: low fat diet with or without probiotic (VSL#3), HFD (became obese mice) with or without probiotic (VSL#3)	t-test, analysis of variance. P<0.05	Probiotic: reduced leptin levels - even in lep ob/ob mice, which means that VSL#3 acts on obesity independently of leptin.	Probiotic: suppressed body weight gain, decreased fat depot size, fat mass and adipocyte size; no difference in food intake; more butyrate production.	No comments.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Yonekura S, 2003	In vitro	Pituitaries, fat and liver tissues from calves and pituitaries cells from male Wistar rats	Intervention	Control, 1, 3 or 10 mM acetate or butyrate	Western blot and RT-PCR triplicate; T-test	Bovine Leptin expression was more than 5 times higher with SCFA than control; it increased in a dose- and time-dependent manner. Butyrate had significant less leptin receptor mRNA expression ($P<0.01$), but not acetate. Rats Butyrate intervention lead to less leptin expression / leptin receptor expression , but unchanged by acetate	-	In bovine pituitary gland leptin expression is regulated by nutrient availability. Besides, acetate only affects bovine, but not rat cells; Butyrate: different species were differently affected regarding leptin expression, bu it decreased LEPRa in both cells
Yonekura S, 2014	In vitro	Bovine preadipocyte	Intervention	Cells were differentiated and then acetate was added in different concentrations (1, 3 or 10 mM)	Western blot and t-test with $p <0.01$, ANOVA and Tukey-Kramer with $p<0.05$	Acetate: cytoplasmic leptin concentration increased in a concentration-dependent manner.	-	Acetate enhance leptin concentration, but not induced adipocyte differentiation

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Yuan H, 2016	Animal	80 male mice	Mechanism	7 control and 73 HFD (induced diabetes and obesity): diabetic control, rice flour with selenium, resistant starch with selenium, resistant starch with more selenium	$P < 0.05$ and for leptin Western blot	RS groups: lower leptin mRNA in adipose tissue and lower serum leptin.	RS: more caecal SCFA, less inflammation; more expression of FFAR2 and 3; Added selenium: no difference of SCFA.	Resistant starch could improve microbiota, including production of SCFA in diabetic. Besides, it can lead to higher expression of FFAR and lower leptin mRNA.

Reference	Sample	Details about sample	SCFA	Methods	Statistical power	Results about leptin	Other important results	Association SCFA / leptin
Zaibi MS, 2010	In vitro	Different types of adipocytes from wild and GPR41 knockout mice	Intervention	Injection of liquid with (0.1 and 0.2mM of acetate, 0.2 and 3mM propionate or 0.2mM butyrate) or without SCFA (control)	One or two-way ANOVA; $p< 0.05$	3mM propionate and 0.2 mM of butyrate markedly stimulated leptin secretion compared to control; 0.2mM of propionate or acetate did not stimulate leptin secretion; 0.2mM of all SCFA significantly lower leptin in knockout mice; Acetate increased leptin secretion from control, but reduced leptin secretion to baseline levels in FFAR3 knockout mice. Butyrate had no effect from either mice. PTX prevented stimulatory effect of propionate on leptin secretion.	More expression of GPR43 in the wild type;	Acetate has an inhibitory effect on leptin secretion in FFAR3 knockout mice; FFAR2 might be related to leptin secretion as butyrate did not affect it and acetate affect it differently on different types of adipocytes.

APPENDIX B

FINAL SEARCH ON PUBMED (MARCH, 13TH 2018)

SCFA related (MeSH terms or Text word) AND Leptin (MeSH terms or Text word)

((((((((((((propionate[Text Word]) OR propionate[MeSH Terms]) OR butyr*[Text Word]) OR butyr*[MeSH Terms]) OR acetate[Text Word]) OR acetate[MeSH Terms]) OR short-chain fatty acids[MeSH Terms]) OR short-chain fatty acids[Text Word]) OR High-amylose maize resistant starch type 2[MeSH Terms]) OR High-amylose maize resistant starch type 2[Text Word]) OR resistant starch[MeSH Terms]) OR resistant starch[Text Word])) AND (((leptin[Text Word]) OR leptin[MeSH Terms]))

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