Chia seed (*Salvia hispanica L.*) effects and their molecular mechanisms on unbalanced diet experimental studies: A systematic review

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Abstract: The aim of this review was to compile evidence and understand chia seed effects on unbalanced diet animal studies and the molecular mechanisms on metabolic biomarker modulation. A systematic review was conducted in electronic databases, following PRISMA recommendations. Risk of bias and quality was assessed using SYRCLE toll and ARRIVE guidelines. Seventeen articles were included. Throughout the studies, chia's main effects are associated with AMPK modulation: improvement of glucose and insulin tolerance, lipogenesis, antioxidant activity, and inflammation. Details about randomization and allocation concealment were insufficient, as well as information about blind protocols. Sample size, chia dose, and number of animals evaluated for each parameter were found to be lacking information among the studies. Based on experimental study data, chia has bioactive potential, and its daily consumption may reduce the risk of chronic disease development, mainly due to the antioxidant, anti-inflammatory, hypoglycemic, and hypolipidemic effects of the seed.

Keywords: alpha linolenic acid, chia seed, dyslipidemia, glucose tolerance, *Salvia hispanica* L

Practical Application: The consumption of chia seeds may improve lipid profile, insulin and glucose tolerance, and reduce risk of cardiovascular disease. Whole seed or its oil presents positive effect, but the effects of chia oil can act faster than the seed.

1. INTRODUCTION

Noncommunicable diseases, such as cardiovascular diseases, cancer, and diabetes, are responsible for three in five deaths in the world (Wang et al., 2016). These diseases are derived from high blood pressure, high cholesterol, and ultimately overweight and obesity (World Health Organization, 2017). Among various factors that may contribute to weight excess, epidemiological studies indicated strong correlation between the consumption of sugar, fructose, and saturated lipids—high content products with obesity. This pattern of food consumption is often termed as the "western diet," characterized by high intakes of fats, animal-source foods, refined carbohydrates, and added sugar. (Popkin, Adair, & Ng, 2011; Popkin, Nielsen, & Bray, 2004).

The "western diet" is considered an unbalanced diet, since the macronutrients distribution is not adequate to human requirements (Institute of Medicine, 2005). Sedentary lifestyle and unbalanced diets lead to overweight (World Health Organization, 2017), which is associated with increased oxidative stress and chronic inflammation, and major biochemical and metabolic changes, which trigger a series of events associated with the development of comorbidities, such as insulin resistance (IR), type 2 diabetes mellitus, and cardiovascular diseases (Pozza & Isidori, 2018).

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In the current context of increased obesity and comorbidities, plant food has been investigated as a source of bioactive compounds that might act as a coadjutant in the prevention and treatment of diseases (Borowska & Brzóska, 2016). The search for mechanisms through which bioactive compounds would improve obese health and decrease comorbidities risk has increased animal studies fed unbalanced diets, since it is a way to mitigate the human food intake pattern that contributes to overweight. Experimental studies involving Rodents are widely used in the nutrition research field since their physiology is similar to human's, including gastrointestinal (Hatton, Yadav, Basit, & Merchant, 2015). Animal studies can be considered an important screening for testing new plant food and their different doses and fractions, attempting a deep investigation into molecular pathways using tissues, which is not possible to accomplish in research involving humans (Chalvon-demersay, Blachier, Tomé, & Blais, 2017). Well-planned animal studies are considered a step before treatment application in clinical trials, which can be more successful when previous data are available to design efficient outcomes (Everitt, 2015).

A plant food alternative is chia seed (*Salvia hispanica* L.), which belongs to Salvia genus (Arctos Specimen Database, 2018), native to northern Guatemala and southern Mexico (Busilacchi et al., 2013), used by Pre-Colombian populations as nutritional food (Ayerza, 2009). This seed is known for its high concentration of alpha linolenic acid (ALA) omega 3 (n-3), dietary fiber, proteins, vitamins, minerals, and phytochemicals, such as phenolic compounds (da Silva et al., 2017; Marineli et al., 2014).

These components present in chia seed have been considered responsible for the improvement of biological markers related to diseases, presenting properties, including anti-inflammatory

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(Hamedi, Jamshidzadeh, Ahmadi, Sohrabpour, & Zarshenas, 2016), antioxidant (Scapin, Schmidt, Prestes, & Rosa, 2016), hypotensive (Toscano et al., 2014), hypoglycemic (Vuksan et al., 2010; Vuksan et al., 2017), and hypocholesterolemic (Toscano, Toscano, Tavares, Oliveira, & Silva, 2015). Though it remains unclear if there is an interaction between them, acting in a synergic way or, if a specific compound is responsible for its health benefits. Besides that, the mechanisms and pathways involved in the chemoprevention of diseases by chia seeds remain unknown.

Thus, the aim of this review was to compile evidence of chia seed effects on unbalanced diet animal studies and to understand the effects and the molecular mechanisms on metabolic biomarker modulation.

2. METHODS

2.1 Protocol and registration

This systematic review was carried out and reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al., 2009). The review has been registered at the PROSPERO (www.crd.york.ac.uk/prospero/): CRD42019119667.

2.2 Literature search

Studies were identified by searching electronic databases: Latin American and Caribbean Center on Health Sciences Information (LILACS), PubMed/MEDLINE, Embase, and Science Direct. The following terms were used to search in each database: ("chia seed" OR "chia seeds" OR "salvia hispanica L.") AND (obesity OR Hyperglycemia OR Inflammation OR Dyslipidemias OR "Insulin Resistance" OR diabetes OR "Cardiovascular Diseases" OR Hypertension OR "metabolic syndrome" OR "nonalcoholic fatty liver disease" OR "nonalcoholic steatohepatitis"). Full search strategy employed in the electronic databases is presented in Table S1. Filters were used, if available, for animal studies. The reason of that choice was based on our purpose of investigating the mechanisms and pathways through which chia seed improves metabolic parameters. The results included original studies conducted with chia seeds and their fractions (oil, fiber, or both), regardless of study duration, dose or concentration used, or experimental design. To be included in this review, the study protocol should have an unbalanced diet group and a control group (standard diet). We excluded studies that used mixtures containing chia seeds, those involving other foods in the same treatment as chia, and those that used chia seed in a healthy diet context.

2.3 Study selection and data collection process

Following the recommendations of PRISMA (Liberati et al., 2009), two researchers independently assessed the eligibility of studies that evaluated the effect of chia consumption. Any discrepancies between reviewers were resolved through consensus. For studies that fulfilled the inclusion criteria (as described in Section 2.1), data extraction was performed by two independently working reviewers who were not blinded to author identity and study origin. Titles and abstracts were examined first, followed by full text screening. The reviewers extracted relevant information from each study: authors, publication date, animal model, chia fraction and dose, duration of the study, study protocol, pathway investigated, and main results. This information was summarized in a standardized model of data extraction.

2.4 Quality assessment

The risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias (SYRCLE RoB) tool (Hooijmans et al., 2014), which is based on the Cochrane Collaboration. The SYRCLE RoB tool was developed to evaluate method quality and to measure the bias involving animal studies (Higgins et al., 2011). The SYRCLE RoB toll considers 10 entries that are related to six types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and others. For each included study, the six bias types were classified as "high" (+, if one or more criteria were not attended), "low" (–, if all criteria were attended), or "unclear" (?, one or more criteria were partly attended).

To increase the strength of the present systematic review, the quality of the included studies was assessed using the Animal Research Reporting of in Vivo Experiment (ARRIVE) guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). The ARRIVE guidelines are intended to standardize and improve the quality of reporting animal studies. It consists of 20 criteria, which evaluate everything from the title to the conclusion of the report with special attention to the methods involved in the study and the discussion of results, as well as the relevance to human biology and the limitations of the study. Each of the included studies was evaluated and for each criterion, "0" or "1" was graded for "not reported" and "reported" information, respectively, and the frequency was calculated.

3. RESULTS AND DISCUSSION

3.1 Included studies and characteristics

The search yielded a total of 165 records from which 17 duplicates were removed. The selection of the remaining studies was made by reading their titles and abstracts and after this step, 19 studies that met the inclusion criteria were retrieved for full-text reading. From the full-text reading, 16 studies attended all the inclusion criteria and were included in this review. During the full-text reading, one study was identified by snowballing adding up to 17 studies included. Most records excluded were review publications, chemical characterization research, studies related to technological properties of chia, and absence of use of chia as study treatment (Figure 1). From 17 included records, only one was conducted with Flanders hybrid rabbits (Sierra, Roco, Alarcon, & Medina, 2015). The others were conducted with rodents: *Wistar* rats (Ayerza & Coates, 2007; Chicco, D'Alessandro, Hein, Oliva, & Lombardo, 2009; Creus, Benmelej, Villafañe, & Lombardo, 2017; Creus, Ferreira, Oliva, & Lombardo, 2016; da Silva et al., 2018; Fortino, Oliva, Rodriguez, Lombardo, & Chicco, 2017; Marineli, Moura, et al., 2015; Marineli, Lenquiste, Moraes, & Maróstica, 2015; Oliva, Ferreira, Chicco, & Lombardo, 2013; Poudyal, Panchal, Waanders, Ward, & Brown, 2012; Poudyal, Panchal, Ward, & Brown, 2013; Poudyal, Panchal, Ward, Waanders, & Brown, 2012), C57BL/6 mice (Fonte-Faria et al., 2019), Swiss mice (de Miranda et al., 2019), and SAMR1/SAMP8 mice (Rui, Yang, Chen, Qin, & Wan, 2018).

A total of nine studies evaluated glucose metabolism, 10 studies evaluated the lipogenesis pathway, five studies analyzed the antioxidant effects, three studies analyzed the inflammation, and one study analyzed the atherosclerosis pathway. Regarding the used fractions, 11 studies used chia seed and the dose varied between 3% and 41.7%. The lowest dose, 3%, was able to increase the high-density lipoprotein cholesterol (HDL-c) of the animals (de Miranda et al., 2019), and the highest dose, 41.7%, was able to increase

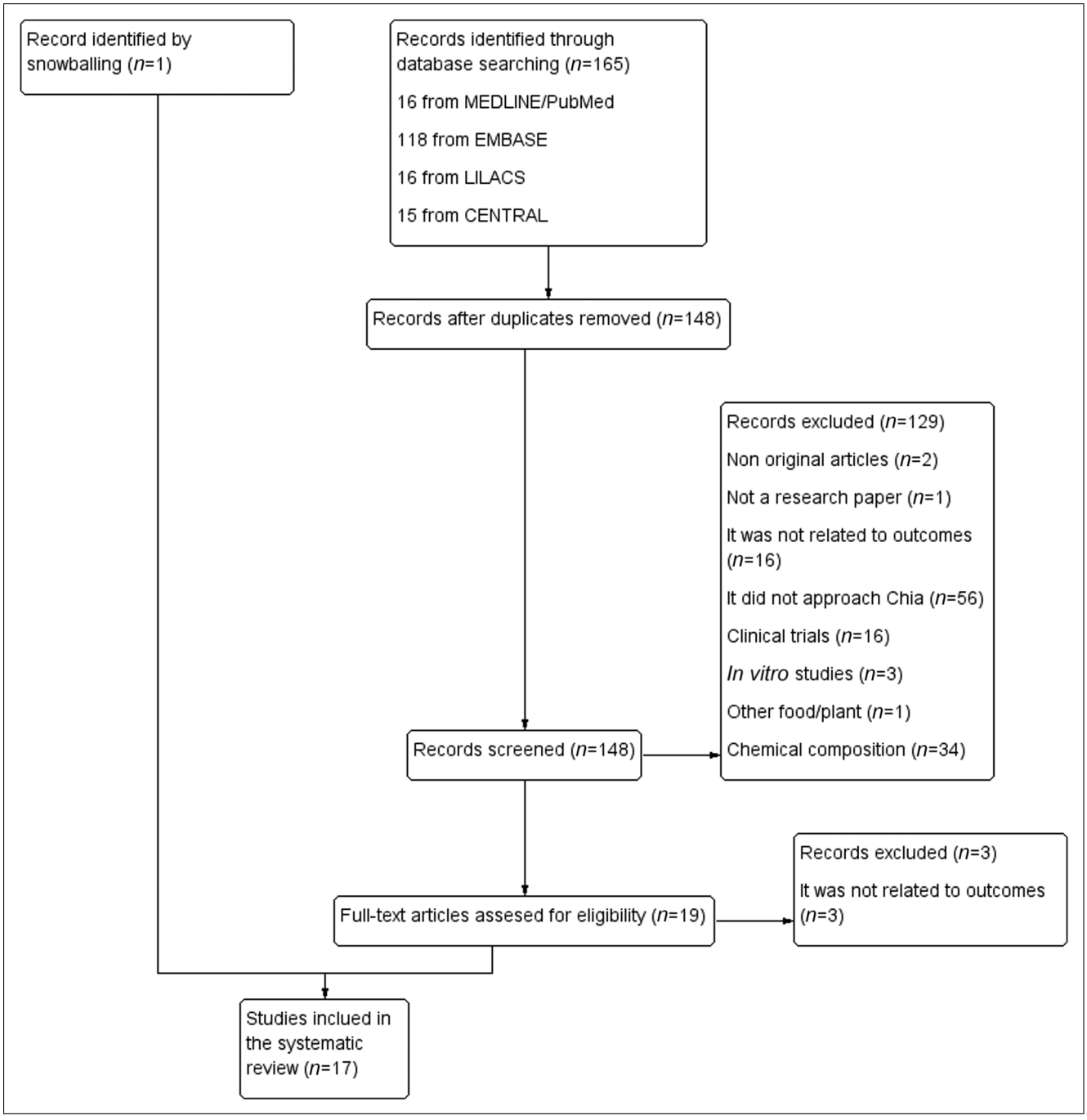

Figure 1–Flowchart of the search and selection process for articles included in the systematic review, according to PRISMA recommendation.

the antioxidant capacity, decrease the inflammatory markers (cytokines and NF-κB expression), and decrease cholesterol (da Silva et al., 2018). The whole seed was offered to animals as flour since particle size acts directly on digestion and metabolic processes (Slavin, 2003). Furthermore, the whole seed flour has every component present in the grain, like endosperm, bran, germ, and coat (Arvola et al., 2007) and the grinding allows bioaccessibility of bioactive antioxidant, such as phenolic acids to cells which increase health benefits (Rosa, Dufour, Lullien-pellerin, & Micard, 2013).

Six studies used chia oil and the doses varied between 0.15% and 10%. The lowest concentration (0.15%) had effects that improved IR, as increased glucose and insulin tolerance induced insulin receptor substrate (IRS) phosphorylation and glucose transporter type 4 (GLUT-4) translocation to plasma membrane, and also reduced the serum fasting insulin levels (Fonte-Faria et al., 2019). Treatment duration varied between 3 and 24 weeks and all studies observed positive effects of chia seeds or oil. Chia seed has the highest known amount of n-3, ranged between 64.8% and 56.9% (Ayerza & Coates, 2011), which may explain the promising results found in animal studies. A summary of each publication is presented in Table 1.

Although there are published clinical trials with chia seeds, this review included only animal studies. Our choice was based on our purpose, which was to investigate the mechanisms and pathways through which chia seed improves metabolic parameters. Most of the clinical trial results are limited to improvement on lipid

Table 1–Characteristics of the animal studies.

References	Animal model	Study protocol	Fraction and dose	Study duration	Pathway	Main results
Fonte-Faria et al. (2019)	C57 BL/6 mice, male	Control ($n = 8$) HFD ($n = 8$) HFD ($n = 8$) followed by HFD + chia oil	Chia oil 0.15%	19 weeks	Glucose metabolism Insulin signaling	↑ Glucose and insulin tolerance ↓ Serum fasting insulin levels ↓ Serum leptin ↓ Serum TG ↑ HDLc ↓ Body fat mass and ↑ Body lean mass ↑ pIRS-1(Tyr)/IRS-1 ↑ GLUT-4 translocation to plasma membrane
da Silva et al. (2018)	*Wistar* rats, male	Control + calcium carbonate ($n = 8$) Control + chia seed ($n = 8$) HFD + calcium carbonate ($n = 8$) HFD + chia seed ($n = 8$)	Chia seed 41.3%	5 weeks	Antioxidant status Inflammation	↓ Total LDLc and VLDLc cholesterol ↑ SOD activity (liver) ↑ CAT (plasma) ↑ PPAR-α ↓ NFκB mRNA expression ↓ NFκB (liver) ↓ TNF and IL-10
de Miranda et al. (2019)	Swiss mice	Control ($n = 6$) Control + chia seed ($n = 6$) HFD ($n = 6$) HFD + chia seed ($n = 6$)	Chia seed 3%	16 weeks	Glucose metabolism Antioxidant status Lipogenesis Inflammation	Did not modify glucose metabolism ↑ HDLc
Rui et al. (2018)	SAMR1 mice SAMP8 mice	SAMR1 LFD ($n = 8$) SAMP8 LFD ($n = 8$) SAMP8 HFD ($n = 8$) SAMP8 HFD + chia seed ($n = 8$)	Chia seed 10%	18 weeks	Glucose metabolism	↓ Insulin (plasma) ↓ HOMA-IR
Creus et al. (2017)	*Wistar* rats, male	Control ($n = 24$) SRD ($n = 24$) SRD ($n = 24$) followed by SRD + chia seed	Chia seed 36.2%	24 weeks	AMPK Glucose metabolism	↓ pAMPK/AMPK ↓ TG, FFA, glucose (plasma) ↑ G-6-P, glycogen (heart muscle) ↑ Hexokinase and PDHa activities (heart muscle) ↑ GLUT-4, IRS-1 (heart muscle) ↓ Collagen and hydroxyproline contents in left ventricle ↓ SBP
Fortino et al. (2017)	*Wistar* rats, male	Male offspring of RD-fed dams: RD-RD (control) Male offspring of SRD-fed dams: SRD-SRD ($n = 30$) SRD-SRD Chia seed ($n = 30$)	Chia seed 20%	20 weeks	Glucose metabolism Lipogenesis	↓ TC, TG (plasma) ↓ n6:n3 ratio ↑ CPT-1 ↓ ACC ↓ Glucose (plasma) ↑ Glucose tolerance ↓ SBP and DBP
Ferreira et al. (2016)	*Wistar* rats, male	Control ($n = 24$, 24 weeks) SRD ($n = 24$, 24 weeks) SRD ($n = 24$) followed by SRD + chia seed	Chia seed 36.2%	12 weeks	Antioxidant status Lipogenesis Inflammation	↓ Epididymal fat and ↑ PPAR-γ (epididymal fat) ↓ TG, FFA, uric acid, glucose, n3:n6 ratio (plasma), and ↑ GIR ↓ TNF, IL-6 (plasma) ↓ TBARS, protein carbonyl groups, XO activity, and ROS ↑ GPx and SOD activity ↑ Nrf2 mRNA

(*Continued*)

Table 1–Continued.

References	Animal model	Study protocol	Fraction and dose	Study duration	Pathway	Main results
Creus et al. (2016)	*Wistar* rats, male	Control ($n = 20$) SRD ($n = 20$) SRD ($n = 20$) followed by SRD + chia seed	Chia seed 36.2%	24 weeks	Lipogenesis	↓Visceral Adiposity Index (%) ↑ GIR ↓TG, FFA, cholesterol, glucose (plasma) ↓ TG, LCA-CoA, DAG, and ↑ PDHa (intramyocardial lipid) ↑ CPT-1 ↑ FAT/CD36 ↓ Cardiac lipotoxicity ↑ Glucose oxidation ↓ SBP
Marineli, Lenquiste et al. (2015)	*Wistar* rats, male	Control ($n = 6$) HFHF ($n = 6$) HFHF followed by HFHF + Chia seed ($n = 6$) HFHF followed by HFHF + Chia oil ($n = 6$) HFHF + Chia seed ($n = 6$) HFHF + Chia oil ($n = 6$)	Chia seed 13.3% Chia oil 4%	Short treatment: 6 weeks Long treatment: 12 weeks	Antioxidant status	↑TAC (plasma) ↑ GSH ↑ GR, GPx (liver and plasma) ↑ FRAP (liver and plasma) ↓ TBARS (plasma) ↓ 8-isoprostane
Marineli, Moura, et al. (2015)	*Wistar* rats, male	Control ($n = 6$) HFHF ($n = 6$) HFHF followed by HFHF + chia seed ($n = 6$) HFHF followed by HFHF + chia oil ($n = 6$) HFHF + chia seed ($n = 6$) HFHF + chia oil ($n = 6$)	Chia seed 13.3% Chia oil 4%	Short treatment: 6 weeks Long treatment: 12 weeks	Antioxidant status Glucose metabolism Lipogenesis	↑ Glucose and insulin tolerance ↑ Expression of HSP70, HSP25 ↓ HSP60 (skeletal muscle) ↑ SOD and GPx ↑ TAC ↑ Expression of PGC-1α ↓ NEFA ↓ ALT e AST
Sierra et al. (2015)	*Flanders* hybrids rabbits	Control ($n = 8$) Chia oil ($n = 8$) Control + cholesterol 1% ($n = 8$) Chia oil + cholesterol 1% ($n = 8$)	Chia oil 10%	6 weeks	Atherosclerosis	↓ TG (plasma) ↑ ALA (plasma) ↑ Endothelium relaxation ↓ Angiotensin II and noradrenaline
Oliva et al. (2013)	*Wistar* rats, male	Control ($n = 6$) SRD ($n = 6$) SRD ($n = 6$) followed by SRD + chia seed	Chia seed	24 weeks	Glucose metabolism	↓ TG, FFA, glucose (plasma) ↑ GIR ↓ Epididymal AT ↓ TG (adipocyte) ↓ FAS, G6PD, malic enzyme ↑ Hexokinase, PDHa (gastrocnemius muscle) ↓ TG, long-chain acyl CoA, and DG (gastrocnemius muscle)
Poudyal et al. (2013)	*Wistar* rats, male	Control ($n = 12$) Control followed by control + chia oil ($n = 12$) HFHF ($n = 12$) HFHF followed by HFHF + Chia oil ($n = 12$)	Chia oil 3%	16 weeks	Lipogenesis	↓ Visceral adiposity (%), abdominal circumference, and retroperitoneal adipose tissue ↓ Liver weight ↓ Lipid content in liver, heart, and skeletal muscle ↓ TC, TG, and NEFA (plasma) ↓ Insulin (plasma) ↑ Glucose and insulin tolerance ↑ n3:n6 (heart, liver, skeletal muscle, retroperitoneal adipose tissue) ↓ AST, LDH, ALP, CK ↑ SBP

(*Continued*)

Table 1–Continued.

References	Animal model	Study protocol	Fraction and dose	Study duration	Pathway	Main results
Poudyal, Panchal, Waanders, et al. (2012)	*Wistar* rats, male	Control ($n = 12$) Control + chia ($n = 12$) HFHF ($n = 12$) HFHF + chia seed ($n = 12$)	Chia seed 5%	24 weeks	Lipogenesis Glucose metabolism	↓Visceral adiposity (%), retroperitoneal and omental adipose tissue ↓ Lipid content in liver and ↑ in heart ↑ TG and ↓ NEFA (plasma) ↑ n3:n6 (plasma and retroperitoneal AT) ↑ Glucose and insulin tolerance ↓ ALT and ↑ ALP ↓ Inflammatory cells in the left ventricle, collagen deposition, diastolic rigidity, fibrosis ↑ SBP
Poudyal, Panchal, Ward, et al. (2012)	*Wistar* rats, male	Control ($n = 12$) Control + Chia seed ($n = 12$) HFHF ($n = 12$) HFHF + Chia seed ($n = 12$) 8 weeks	Chia seed 5%	8 weeks	Lipogenesis Glucose metabolism	↓ Visceral adiposity (%), body fat (%), abdominal circumference, retroperitoneal and omental adipose tissue, and ↑ total body lean mass ↓ Lipid content in skeletal muscle ↑ Lipid content in heart ↑ Glucose and insulin tolerance ↓ Uric acid, LDH, CRP, AST ↑ TG, ALP, and n3:n6 ratio (plasma) ↓ Liver fibrosis ↑ SBP
Chicco et al. (2008)	*Wistar* rats, male	Experimental design 1: Control ($n = 24$) SRD ($n = 24$) SRD + chia seed ($n = 24$) Experimental design 2: Control ($n = 24$) SRD ($n = 72$) divided into three subgroups: 1. immediately killed ($n = 24$) 2. SRD ($n = 24$) 3. SRD + chia seed ($n = 24$)	Chia seed 36.2%	Experimental design 1: 3 weeks Experimental design 2: 20 weeks	Lipogenesis	↓Epididymal and retroperitoneal AT ↓ TG, NEFA, TC (plasma) ↓ TG (liver) ↓ Glucose ↑ Glucose tolerance ↑ n3 total ↓ n6:n3 ratio (plasma)
Ayerza & Coates (2007)	*Wistar* rats, male	Control ($n = 8$, 4 weeks) Whole chia seed ($n = 8$) Ground chia seed ($n = 8$) Chia oil ($n = 8$)	Chia seed: 16% Chia oil: 5.34%	4 weeks	Lipogenesis	↓ TG, total SFA (plasma) ↑ HDLc and n3 (plasma)

↑, increase; ↓, decrease; ACC, acetyl-CoA carboxylase; ALA, alpha linolenic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMPK, adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; AT, adipose tissue; CAT, catalase; CK, creatinine; CPT-1, carnitine-palmitoyl transferase-1; CRP, C-reactive protein; DBP, diastolic blood pressure; DG, diacylglyceride; FAS, fatty acid synthase; FAT/DC36, fatty acid translocase; FFA, free fatty acid; FRAP, ferric reducing ability of plasma; G-6-P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GIR, glucose infusion rate; GLUT-4, glucose transporter type 4; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; HDLc, high-density lipoprotein; HFD, high-fat diet; HOMA-IR, homeostasis model assessment: insulin resistance; HSP25, heat shock protein 25; HSP60, heat shock protein 60; HSP70, heat shock protein 70; IL-10, interleukin 10; IL-6, interleukin 6; IRS-1, insulin receptor substrate 1; LCA-CoA, long-chain acyl-CoA; LDH, lactate dehydrogenase; LDLc, low-density lipoprotein; n3, omega 3; n6, omega 6; NEFA, nonesterified fatty acid; NF-κB, factor nuclear kappa B; Nrf2, nuclear factor (erythroid-derived 2)-like 2; pAMPK, phosphorylation of adenosine monophosphate-activated protein kinase; PDHa, pyruvate dehydrogenase E1 component subunit alpha; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator; pIRS-1(Tyr)/IRS-1, phosphorylation on Tyr989 of IRS-1; PPAR-α, peroxisome proliferator-activated receptor alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; RD, reference diet; RD-RD, offspring from dams fed a reference diet (RD) and fed the RD after weaning; ROS, reactive oxygen species; SAMP8 HFD, enescence accelerated mouse - fed a high-fat diet; SAMP8 LFD, senescence accelerated mouse-acknowledgments fed a low-fat diet; SAMR1 LFD, senescence-accelerated mouse-resistant 1 – fed a low-fat diet; SBP, systolic blood pressure; SD, standard diet; SFA, saturated fatty acid; SOD, superoxide dismutase; SRD, sucrose rich diet; SRD-SRD, offspring from SRD-fed dams fed an SRD after weaning; SRD-SRDC, offspring from SRD-fed dams fed an SRD+chia after weaning; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substance; TC, total cholesterol; TG, triacylglycerol; TNF-α, tumor necrosis factor alpha; VLDLc, very low-density lipoprotein; XO, xanthine oxidase.

profile (Nieman et al., 2009; Nieman et al., 2012; Toscano et al., 2014; Toscano et al., 2015; Vuksan et al., 2017), oxidative stress (Toscano et al., 2014; Vuksan et al., 2007; Vuksan et al., 2017), blood pressure (Toscano et al., 2014; Vuksan et al., 2007), and weight loss (Toscano et al., 2015; Vuksan et al., 2017), but all these parameters were not reproducible. This lack of major results may be due to feasible doses of chia used in these studies, which can be considered low when compared to doses offered to animals (20 g/kg body weight) (Creus et al., 2017). The amount of chia seed (as food) expected to improve metabolic parameters may be too high for human consumption.

3.2 Impact of chia on metabolic and associated disorders

3.2.1 Glucose metabolism. The introduction of chia to rats fed with an unbalanced diet improved glucose tolerance and insulin sensitivity (Chicco et al., 2009; Creus et al., 2016; Creus et al., 2017; Ferreira, Alvarez, Illesca, Giménez, & Lombardo, 2016; Fonte-Faria et al., 2019; Fortino et al., 2017; Marineli, Moura, et al., 2015; Marineli, Lenquiste, et al., 2015; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012; Rui et al., 2018). These effects were confirmed by additional data of chia seed intake, such as increased expression of heat shock proteins 25 (HSP25) and HSP70 in soleus muscle (Marineli, Moura, et al., 2015), increased insulin-stimulated phosphorylation on IRS-1 and GLUT-4 translocation on gastrocnemius (Fonte-Faria et al., 2019) and heart (Creus et al., 2017), increased adenosine monophosphate-activated protein kinase(AMPK) phosphorylation on gastrocnemius (Fonte-Faria et al., 2019), cardiac muscles (Creus et al., 2017), epididymal, and subcutaneous adipose tissues (Rui et al., 2018).

AMPK is one of the most important proteins involved to the cellular energy balance and it works as sensor of energy-deprivation. Its decrease leads to noticeable effects on animal energy metabolism, among which are the oxidative pathways of glucose and lipids (Nelson & Cox, 2014). It is already well established that diets high in fat and sucrose (or both) reduce AMPK (Lindholm et al., 2013; Yang, Miyahara, Takeo, & Katayama, 2012) and HSP expression (Chung et al., 2008). Therefore, chia seed and its compounds present a promising role in energetic metabolism since animals fed unbalanced diets, when fed chia, had AMPK phosphorylation restored as control animals. Besides that, chia modulates other markers, including carnitine palmitoyltransferase 1 (CTP-1) (Creus et al., 2017; Fortino et al., 2017), peroxisome proliferator-activated receptor-γ coactivator 1-alpha (PGC-1α) and HSP expression (Marineli, Moura, et al., 2015), signaling improvement in mitochondrial activity pattern, fatty acid oxidation, adipogenesis control, and prevention of the overexpression of inflammatory mediators (Creus et al., 2016; da Silva et al., 2018; Musch, Kapil, & Chang, 2004).

From the included studies, we hypothesize that chia and its fractions mitigate obesity-induced insulin sensitivity by regulating AMPK and IRS-1 phosphorylation, which improve GLUT-4 translocation and increase hexokinase and glucose 6-phosphate enzymatic activity (Chicco et al., 2009; Creus et al., 2017; Ferreira et al., 2016; Fortino et al., 2017; Marineli, Moura, et al., 2015; Oliva et al., 2013; Poudyal, Panchal, Ward, et al., 2012), which would restore glucose utilization as an energetic fuel. This hypothesis is supported by the increase in CPT-1 activity (Creus et al., 2016), PGC-1α expression, and peritoneal glucose tolerance as a response to consumption of chia seed and oil (Marineli, Moura, et al., 2015) (Figure 2).

3.2.2 Lipid profile and lipolysis. The consumption of chia improved the lipid profile in animals fed nutritionally inadequate diets (high in simple carbohydrates like sucrose and fructose, high in saturate fat, or both). These results are related to improving plasma cholesterol profile (Creus et al., 2016; da Silva et al., 2018; de Miranda et al., 2019; Fonte-Faria et al., 2019; Poudyal et al., 2013; Sierra et al., 2015) and decreasing plasma triglycerides (Ayerza & Coates, 2007; Chicco et al., 2009; Creus et al., 2016; Creus et al., 2017; Fonte-Faria et al., 2019; Fortino et al., 2017; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal et al., 2013; Sierra et al., 2015). Additionally, chia seed increased the plasmatic levels of n-3 and α-linoleic (n-6) fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), n-3/saturated fatty acids, and n-3/n-6 ratio (Ayerza & Coates, 2007; Chicco et al., 2009; Creus et al., 2017; Fortino et al., 2017; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Sierra et al., 2015).

Among plant foods, chia seed is the major source of n-3 (Ayerza, 2009; Ayerza & Coates, 2005; da Silva et al., 2017) and despite being the primary fatty acid of the n-3 pathway, its conversion to EPA and DHA has been widely held as inefficient (Ratnayake & Galli, 2009). Studies with chia seed as a source of ALA have been demonstrating that its physiological effects are different than that produced by EPA and DHA in rats with metabolic syndrome. They also showed that although ALA was not efficient in reducing total body fat (Poudyal, Panchal, Waanders, et al., 2012; Poudyal et al., 2013), it has induced fat redistribution away from the abdominal area (Fonte-Faria et al., 2019; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012; Poudyal et al., 2013), decreasing the risk of cardiovascular diseases.

Moreover, the modulation of glucose metabolism markers by chia seed had impact on lipogenesis, evidenced by the increase of peroxisome proliferator-activated receptor (PPAR) (Creus et al., 2016; da Silva et al., 2018), improvement in serum lipid profile, reduction of visceral adiposity, and decrease of epididymal adipose tissue weight (Ayerza & Coates, 2007) (Figure 2; Chicco et al., 2009; Creus et al., 2016; Creus et al., 2017; Ferreira et al., 2016; Fortino et al., 2017; Oliva et al., 2013; Sierra et al., 2015).

Regarding lipogenesis, rats fed a high-fat diet (HFD) had impaired recruitment of FAT/CD36 by insulin as well as CTP-1 activity (Creus et al., 2016) and PPAR-α protein mass (Creus et al., 2016; da Silva et al., 2018). These findings are related to chronic high exposure to fatty acids provided by HFD, disrupting the balance between lipid oxidation-storage. The recruitment of FAT/CD36 by insulin is a key mechanism for beta-oxidation and for restoring mitochondrial activity. Chia seed was able to revert this condition by recruiting FAT/CD36 to the sarcolemma through insulin signaling (Creus et al., 2016), increasing PPAR-α (da Silva et al., 2018), increasing PGC1-α expression (Marineli, Moura, et al., 2015), and decreasing visceral adiposity (Creus et al., 2016; Ferreira et al., 2016; Poudyal, Panchal, Ward, et al., 2012; Poudyal et al., 2013).

Our review allowed us to develop a hypothesis about how chia seed can improve lipid biomarkers. Unbalanced diets induce insulin intolerance and this is a key event for lipogenesis. Chia seed reversed abnormal glucose homeostasis and peripheral insulin insensitivity (Chicco et al., 2009; Creus et al., 2016; Creus et al., 2017; Ferreira et al., 2016; Fonte-Faria et al., 2019; Fortino et al., 2017; Marineli, Moura, et al., 2015; Marineli, Lenquiste, et al., 2015; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012;

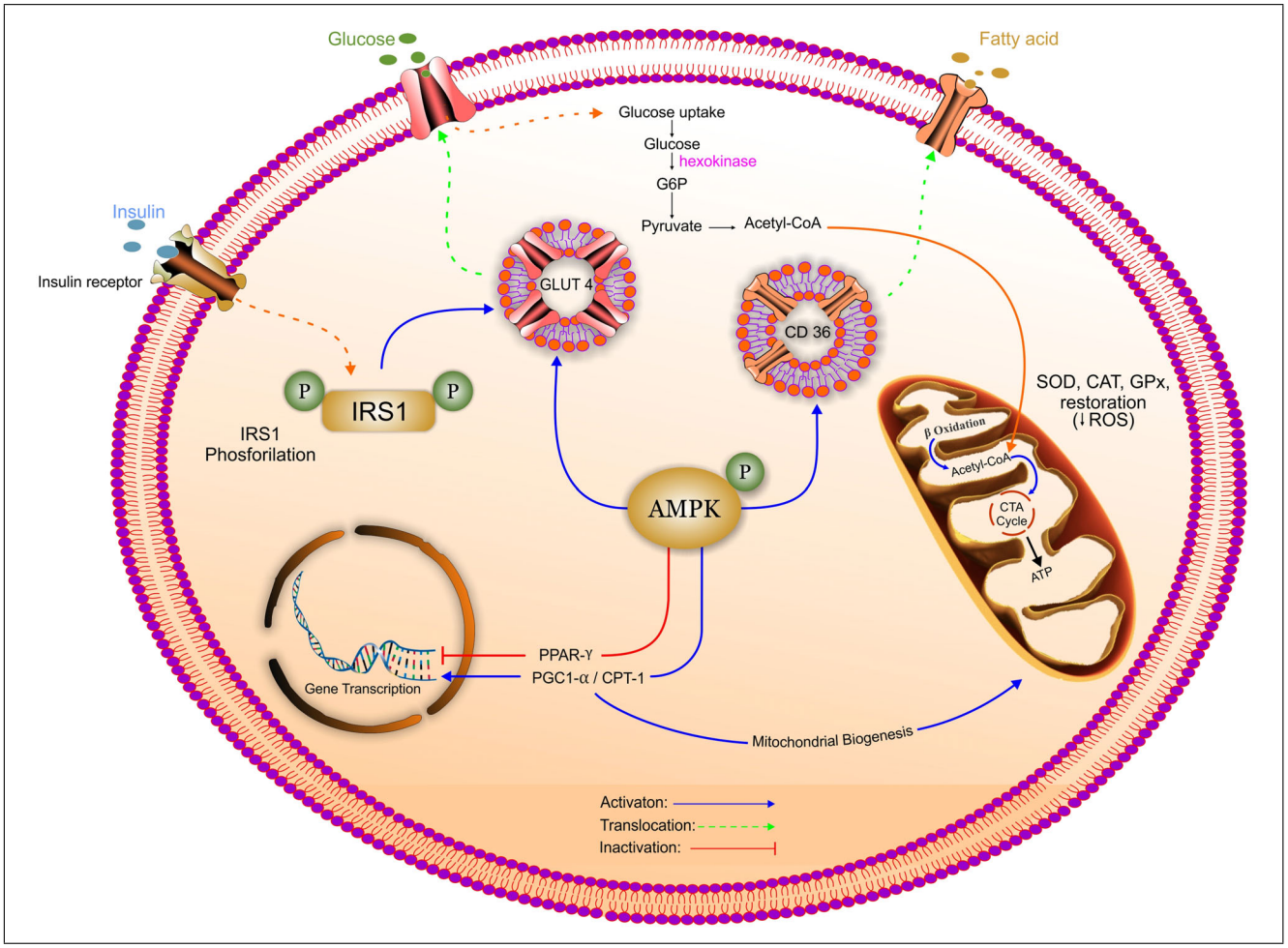

Figure 2–Hypothesis of chia's seeds mechanism of action based on unbalanced diet experimental studies. AMPK, AMP-activated protein kinase; CTA, tricarboxylic acid; CTP-1, carnitine palmitoyl transferase I; G6PD, glucose 6 phosphate; GPx, gluthatione peroxidase; IRS1, insulin receptor substrate 1; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SOD, superoxide dismutase.

Poudyal, Panchal, Ward, et al., 2012; Rui et al., 2018) and insulin induced the translocation of FAT/CD36 (Creus et al., 2016) to the plasmatic membrane. Our hypothesis is that the normalization of insulin response is one of the most important events involved in chia seed improvement on lipogenesis, recovering beta-oxidation and balancing fuel utilization. Thus, the normalization of insulin response by chia seed may reverse triggers for lipogenesis.

3.2.3 Impact of chia on oxidative stress and inflammation. Several authors described chia's effects on decreasing oxidative stress by increasing total antioxidant capacity, restoring antioxidant enzymes (da Silva et al., 2018; Marineli, Moura, et al., 2015; Marineli, Lenquiste, et al., 2015), and decreasing reactive oxygen species (ROS) and lipid peroxidation (Ferreira et al., 2016; Marineli, Lenquiste, et al., 2015). The decrease of the plasma lipid peroxidation may be connected to hypoglycemic effect of chia that is associated with the decrease of low-density lipoprotein oxidation, probably due to a reduction on its plasmatic levels (Marineli, Lenquiste, et al., 2015). With the exception of de Miranda et al. (2019), all the studies included in this review that evaluated glucose metabolism, plasma lipids, or its peroxidation and ROS production found simultaneously glycemia decrease and plasmatic lipid level improvement (Creus et al., 2016; Creus et al., 2017; Ferreira et al., 2016; Fonte-Faria et al., 2019; Fortino et al., 2017; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal et al., 2013). Three other papers showed decrease in peroxidation markers (Ferreira et al., 2016; Marineli, Moura, et al., 2015; Marineli, Lenquiste, et al., 2015).

Compounds in chia, such as phenolic acids (rosmarinic acid, caffeic acid, danshensu, chlorogenic acid, quercetin, myricetin, and kaempferol) and lipophilic compounds (carotenoids, tocopherols, phospholipids, and ALA), were associated with antioxidant effects (da Silva et al., 2017; Ixtaina et al., 2010; Oliveira-Alves et al., 2017; Reyes-Caudillo, Tecante, & Valdivia-López, 2008). Although antioxidant activity and phenolic compound quantities between chia seeds and oil are different (Oliveira-Alves et al., 2017), studies have highlighted similar effects of both seed and oil in the modulation of oxidative stress (Marineli, Lenquiste, et al., 2015). The beneficial effect demonstrated for both fractions may be due to interactions between the chemical components in the seed, and synergistic activity between the lipophilic compounds in chia oil. The variability observed between the studies may be due to the distinct composition of chia from different locations and cultivation conditions (da Silva et al., 2017).

Regarding the effect of chia seed on inflammation, some studies showed reduction on inflammation biomarkers (Creus et al.,

Table 2–Risk of bias from experimental studies.

References	Marineli, Moura, et al. (2015)	Marineli, Lenquiste, et al. (2015)	Oliva et al. (2013)	Poudyal, Panchal, Waanders, et al. (2012)	Creus et al. (2017)	Creus et al. (2016)	Chicco et al. (2008)	Sierra et al. (2015)	Ayerza; Coates (2007)	Fortino et al. (2017)	Poudyal, Panchal, Ward, et al. (2012)	Ferreira et al. (2016)	Poudyal et al. (2013)	Rui et al. (2018)	de Miranda et al. (2019)	da Silva et al. (2018)	Fonte-Faria et al. (2019)	Percentage (%)
(1) Title	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	94.1
(2) Abstract	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	88.2
Introduction																		
(3) Background information	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(4) Primary and secondary objectives	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	94.1
Methods																		
(5) Ethical statement	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(6) Study design, allocation concealment, blinding, and randomization	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(7) Experimental procedure	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	94.1
(8) Experiment animal details, including species, gender, age, weight, and source	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(9) Housing and husbandry conditions	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(10) Sample size	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	88.2
(11) Allocation of animals to experimental groups	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(12) Experiment outcomes	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(13) Statistical analysis	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	94.1
Results																		
(14) Baseline data	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(15) Number of animals analyzed	1	1	0	0	0	0	0	1	1	1	0	1	1	0	1	0	1	52.9
(16) Outcomes and estimation, results for each animal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(17) Adverse events (details of all important adverse)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discussion																		
(18) Interpretation, scientific implications, and study limitations	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100
(19) Generalizability and translation, relevance to human biology	1	1	0	1	0	1	0	0	1	0	0	1	1	1	1	1	1	64.7
(20) Funding	1	1	0	1	0	1	0	1	1	0	1	0	0	1	1	1	1	64.7

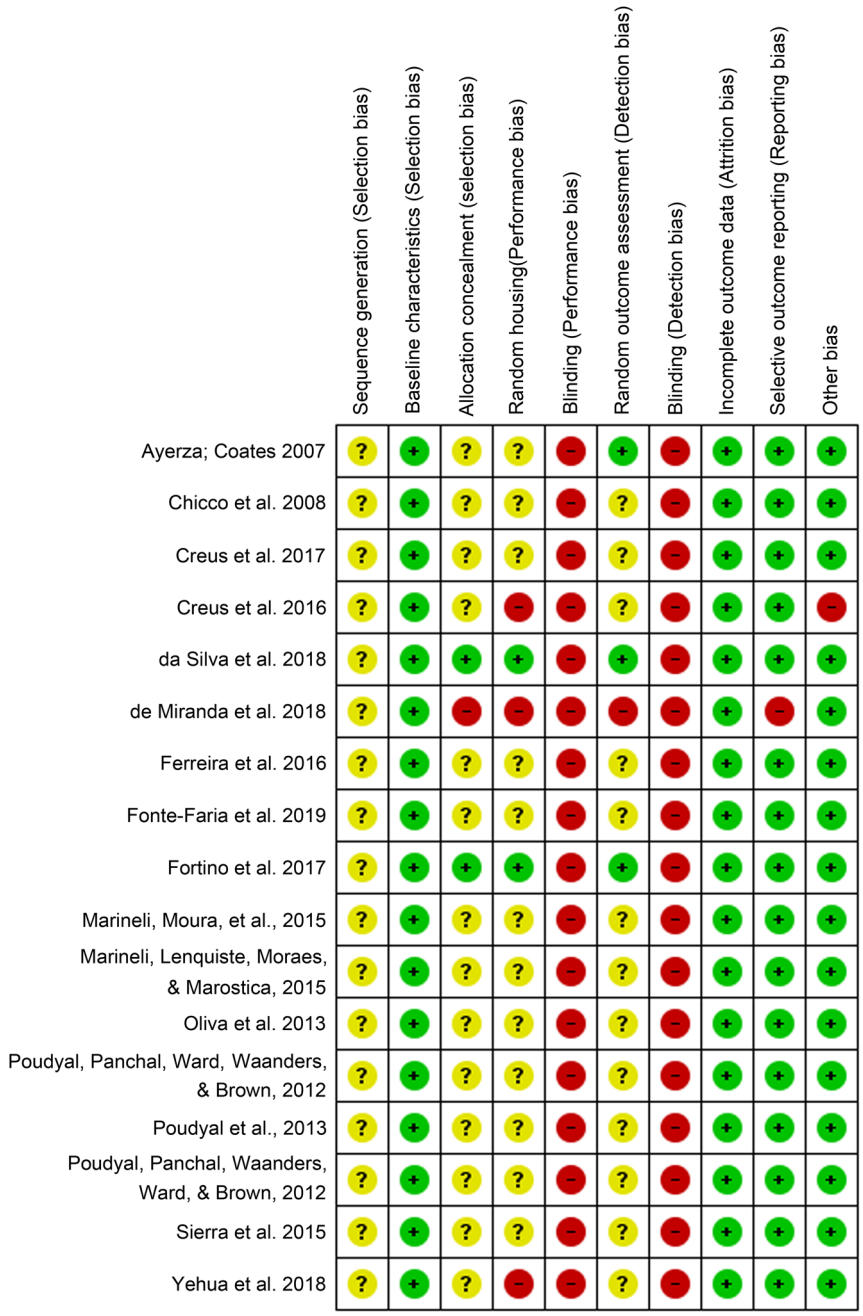

Figure 3–Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

2016; Creus et al., 2017; da Silva et al., 2018; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012) or on associated ones, such as glutamic-oxalacetic transaminase, alanine aminotransferase (Marineli, Moura, et al., 2015; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012; Poudyal et al., 20132012), liver fibrolisis (Poudyal, Panchal, Waanders, et al., 2012), and HSP72 upregulation, which is associated with preventing the overexpression of inflammatory mediators (Moura, Lollo, Morato, & Amaya-Farfan, 2018). All of them interestingly were associated with other improvements, such as glucose and insulin tolerance and lipogenesis control. This finding strengthens our previous hypothesis about insulin activity normalization and positive effects of chia seed on health.

3.3 Impact of chia's bioactive compounds on metabolic and associated disorders

All studies included in this review mentioned the bioactive compounds of chia seed as potential in improving metabolic disorders developed by unbalanced diets. However, none of them are able to point out a compound as responsible for a specific action. Previous studies using other food sources or isolated bioactive compounds evidenced that phenolic compounds (for example, quercetin, chlorogenic acid, caffeic acid, and rosmarinic acid), dietary fiber, fatty acid (for example, ALA), and others play a role in repairing health conditions triggered by unbalanced diets (Gonzalez-Manan et al., 2012; Pal & Ghosh, 2012; Sadeghi, Seyyed Ebrahimi, Golestani, & Meshkani, 2017). Nevertheless, regarding chia seed, it is hard to link the effect on metabolism to

one specific compound. Even the studies that used fractions like oil or fiber cannot discern which "compound" is associated with that action.

The fractions of the seed have different components that result in distinct actions. Apparently hydrolyzed extracts of chia as well as extracts from the seed and its fiber present higher antioxidants (Oliveira-Alves et al., 2017), and flavonoid bioaccessibility may be impaired by the seed's fat (Pellegrini et al., 2018). Nevertheless, studies conducted with chia oil presented improvement in inflammation (Poudyal, Panchal, Waanders, et al., 2012) and restored the antioxidant system (Marineli, Lenquiste, et al., 2015). Studies that compared chia's fraction during different times showed that chia oil presents a faster action than chia seed (Marineli, Moura, et al., 2015; Marineli, Lenquiste, et al., 2015), but more studies must be conducted to confirm it. Although chia oil seems to be superior to the seed, the extraction methods should be observed in order to avoid losses regarding antioxidant compounds. Özcan, Al-Juhaimi, Ahmed, Osman, and Gassem (2019a) showed that chia oil obtained from roasted seeds presents lower content of α-Tocopherol, $\beta + \gamma$-Tocopherol, δ-Tocopherol, β-Tocotrienol, and γ-Tocotrienol compared with nonroasted seeds, regardless of the method used to obtain the oil: cold press or Soxhlet extraction. Recent data showed that heat acts negatively on physical-chemical and bioactive properties of chia oil, where the content of fatty acids and phenolic compounds was decreased by microwave roasting (Özcan, Al-Juhaimi, Ahmed, Osman, & Gassem, 2019b).

Therefore, it still remains unclear if there is a specific bioactive compound or the synergism of them is implicated in the improvement of biomarkers evidenced by the studies. None of them make a direct association with a certain compound with the results obtained. However, most of the studies mentioned that the major action of chia on health conditions is due to the improvement of glucose uptake, its oxidation and restored tissue sensibility to insulin, and its regulation of gene expression (PPARα, CTP-1, and PGC1-α) related to lipogenesis and mitochondrial activity.

Based on the few studies that evaluated the effects of chia on metabolic pathways, we hypothesized that chia compounds increase AMPK expression, which increases the PGC1-α and CTP-1, increasing the expression of PPARα, reducing the lipogenesis, increasing mitochondrial activity, and consequently fatty acid oxidation. Therefore, the AMPK together with IRS stimulates the translocation of GLUT-4 to plasmatic membrane and allows the entrance of glucose inside the cells, reducing the IR. Moreover, AMPK allows the translocation of FAT/CD36 to the sarcolemma in muscle cells, facilitating the FA oxidation by mitochondria. Chia's effects on restoring antioxidant defense may come from the improvement on glucose tolerance, from the reduction of oxidative stress, or the improvement on mitochondrial dysfunction, but it is unknown how chia seed is able to increase these antioxidative enzymes (Figure 2).

3.4 Reporting quality and risk of bias

Among the 17 studies evaluated, one study did not show the dose of chia administered to the animals (Chicco et al., 2009). None of them discussed potential adverse effects of the doses and/or compounds used in the intervention, as well as insufficient information on the limitations of the study, especially concerning extrapolation of data to humans (Creus et al., 2017; Fortino et al., 2017; Oliva et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012; Poudyal et al., 2013) (Table 2; Sierra et al., 2015). Our findings are consistent to Kilkenny et al. (2009), who claim that there is a lack of important information regarding experimental and statistics methods applied in research regarding animals and in publications.

Moreover, about the risk of bias (Figure 3), none of the studies reported about blinding the investigators involved in the research. Only one study (da Silva et al., 2018) reported details about the animal's randomization to groups. Sixteen studies failed to report information about allocation concealment strategies. The lack of information reported in studies may reveal significant shortcomings on designing animal studies, which in turn may impair the scientific community to obtain reliable data from previous studies, generating a negative effect on laboratory research. Our data about lack of blinding are consent with findings demonstrating little blinding in experimental research (Holman, Head, Lanfear, & Jennions, 2015). Selection and measurement bias can be solved with randomization and blinded assessment of outcome (Macleod et al., 2015), since they are related to the randomness of outcomes. We suggest researchers involved in animal studies to follow the ARRIVE guidelines to avoid misinformation in their reports.

4. CONCLUSIONS

This review supports the prospective use of chia in the prevention and treatment of comorbidities associated with unbalanced diets. Despite the limitations in extrapolating the results to humans, we consider chia seed a potential bioactive food, as the realistic consumption of chia seeds and oil could be able to prevent and attenuate metabolic changes. We highlight the lack of data about which compound would be responsible to stimulate or downregulate the pathways discussed in this review as well as the dose that would present an efficient and secure effect on human health. We reinforce the need of future clinical studies that consider the dose of chia seed consumed daily and the seed fraction that best impacts health. Thus, the biological effects of the seed and its components can be clinically confirmed and may represent a dietary strategy to prevent and treat chronic health problems. The use of tools for quality assessment identified methodological gaps that suggest operational improvements on running experimental research for future high-quality controlled trials.

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CONFLICT OF INTEREST

The authors state they have no conflict of interest.

AUTHOR CONTRIBUTION

Bárbara N. Enes researched prior studies, evaluated each study, compiled data, interpreted the results, and drafted the manuscript. Luiza P. D. Moreira researched prior studies, evaluated each study, interpreted the results, and drafted the manuscript. Bárbara P. Silva compiled data and drafted the manuscript. Mariana Grancieri drafted the manuscript and reviewed the manuscript. Haira G. Lúcio researched prior studies and compiled data. Vinícius P. Venâncio drafted and reviewed critically the manuscript. Susanne U. Mertens-Talcott and Carla O.B. Rosa reviewed critically the manuscript. Hércia S.D. Martino reviewed critically the manuscript and approved the final and revised version. All authors have read and approved the final manuscript.

NOMENCLATURE

ACC	acetyl-CoA carboxylase
ALA	alpha linolenic acid
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMPK	adenosine monophosphate-activated protein kinase
ARRIVE	animal research reporting of in vivo experiment
AST	aspartate aminotransferase
AT	adipose tissue
CAT	catalase
CK	creatinine
CPT-1	carnitine-palmitoyl transferase-1
CRP	C-reactive protein
DBP	diastolic blood pressure
DG	diacylglyceride
FAS	fatty acid synthase
FAT/DC36	fatty acid translocase
FFA	free fatty acid
FRAP	ferric reducing ability of plasma
G-6-P	glucose-6-phosphate
G6PD	glucose-6-phosphate dehydrogenase
GIR	glucose infusion rate
GLUT-4	glucose transporter type 4
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
HDLc	high-density lipoprotein cholesterol
HFD	high-fat diet
HOMA-IR	homeostasis model assessment: insulin resistance
HSP25	heat shock protein 25
HSP60	heat shock protein 60
HSP70	heat shock protein 70
IL-10	interleukin 10
IL-6	interleukin 6
IRS-1	insulin receptor substrate 1
LCA-CoA	long-chain acyl-CoA
LDH	lactate dehydrogenase
LDLc	low density lipoprotein
n3	omega 3
n6	omega 6
LILACS	Latin American and Caribbean Center on Health Sciences Information
NEFA	nonesterified fatty acids
NF-κB	factor nuclear kappa B
Nrf2	nuclear factor (erythroid-derived 2)-like 2
pAMPK	phosphorylation of adenosine monophosphate-activated protein kinase
PDHa	pyruvate dehydrogenase E1 component subunit alpha
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator
pIRS-1(Tyr)/IRS-1	phosphorylation on Tyr989 of IRS-1
PRISMA	preferred reporting items for systematic reviews and meta-analysis
PPAR-α	peroxisome proliferator-activated receptor alpha
PPAR-γ	peroxisome proliferator-activated receptor gamma
RD	reference diet
RD-RD	offspring from dams fed a reference diet (RD) and fed the RD after weaning
ROS	reactive oxygen species
SAMP8 HFD	senescence accelerated mouse – fed a high-fat diet
SAMP8 LFD	senescence accelerated mouse – fed a low-fat diet
SAMR1 LFD	senescence accelerated mouse – resistant 1 – fed a low-fat diet
SBP	systolic blood pressure
SD	standard diet
SFA	saturated fatty acid
SOD	superoxide dismutase
SRD	sucrose rich diet
SRD-SRD	offspring from SRD-fed dams fed an SRD after weaning
SRD-SRDC	offspring from SRD-fed dams fed an SRD+chia after weaning
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
TAC	total antioxidant capacity
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TG	triacylglycerol
TNF-α	tumor necrosis factor alpha
VLDLc	very low-density lipoprotein
XO	xanthine oxidase

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Search strategy for different databases