DIETARY PULSES AS AN ACCESSIBLE MEANS TO IMPROVE THE GUT MICROBIOME, INFLAMMATION, AND APPETITE CONTROL IN INDIVIDUALS WITH OBESITY

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Abstract

Interest in the gut bacterial community residing in the human intestine, otherwise known as the gut microbiota, has exploded in recent years. The gut microbiome has been linked to chronic diseases such as obesity, suggesting interventions that target the microbiome may be useful in treating obesity and its complications. Dietary pulses (*e.g.*, common beans) are composed of nutrients and compounds that possess the potential to modulate the gut bacteria composition and function which can in turn improve appetite regulation and chronic inflammation in obesity. This review summarizes the current state of knowledge regarding the connection between the gut microbiome and obesity, appetite regulation, and systemic and adipose tissue inflammation. More specifically, it highlights the efficacy of interventions employing dietary common beans as a means to improve appetite regulation and inflammation in obesity in both rodent models and in humans. Collectively, results presented and discussed herein provide insight on the gaps in knowledge necessary for a comprehensive understanding of the potential of beans as a treatment for obesity while highlighting what further research is required to gain this understanding.

Keywords: Gut microbiota, appetite, inflammation, dietary pulses.

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List of Abbreviations

LWO – living with obesity
SCFAs – short-chain fatty acids
NDCs – non-digestible carbohydrates
F/B – Firmicutes/Bacteroidetes
BMI – body mass index
HFD – high-fat diet
GLP-1 – glucagon-like peptide 1
PYY – peptide YY
NPY - neuropeptide Y
AgRP - agouti-related peptide
POMC - proopiomelanocortin
LFD – low-fat diet
GPR – G-protein coupled receptor
NF – nuclear factor
IL - interleukin
TNF - tumor necrosis factor
MCP - monocyte chemotactic protein
hs-CRP – high sensitivity C-reactive protein
LPS – lipopolysaccharide
TLR4 – toll-like receptor 4
EAT – epididymal adipose tissue
STAT3 - signal transducer and activator of transcription 3
IFN - interferon
MIP - macrophage inflammatory protein

Chapter 1: Introduction

Obesity is a chronic disease[1] that has been linked with alterations in the gut microbiome and appetite regulation, as well as chronic low-grade systemic inflammation[2]. Most current obesity treatments are weight-centered[3] despite evidence showing that weight loss is very unlikely to be sustained in the long-term[4]. Furthermore, improvements in health and reductions in mortality risk can occur in the absence of weight loss[5]. Obesity Canada has expressed the need for more research focused on improving health outcomes rather than weight loss [6]. Dietary pulses are non-oilseed legumes[7] and have become a food of interest because of their potential health benefits. In addition to being a high source of plant protein, pulses are high in non-digestible carbohydrates (NDCs) and polyphenols[7]. The fermentation of NDCs and polyphenols by gut bacteria results in the production of metabolites, such as short-chain fatty acids (SCFAs) and phenolic compounds, that have been found to alter the gut microbiome and improve appetite regulation and inflammation[8,9]. Additionally, common beans (*Phaseolus* vulgaris) are a pulse type with higher fibre[10] and polyphenol[11] content relative to other pulse types. Therefore, common beans could potentially act as a health-centered intervention in the management of obesity and some of its related co-morbidities.

Research on the effect of common beans on the gut microbiome, appetite regulation, and inflammation in obesity is limited. There is still need for a comprehensive study that investigates the relationship between bean consumption and the gut microbiome, appetite, and inflammation in individuals with obesity; this was the aim of the original proposed project for this thesis, but due to COVID-19 restrictions this was not possible. Instead, a narrative literature review was conducted with the goal of summarizing the current state of the literature surrounding this topic.

Both animal and human models of obesity were reviewed in order to gain a more extensive understanding on the full effects beans have on the gut microbiome, appetite, and inflammation.

Following the introduction of this review article, there is a brief description of dietary pulses and the nutritional composition of common beans. The remainder of the review is separated into sections for the gut microbiome, appetite regulation, and inflammation. To facilitate the understanding of the relevance of each topic, a summary of the relationship each variable (i.e., gut microbiome, appetite control, and inflammation) has with obesity and the proposed mechanisms by which common beans interact with each variable is provided. A non-exhaustive list of studies using animal models of diet-induced obesity and short- and long-term studies with individuals living with obesity (LWO) are summarized for each topic. Data taken from studies measuring the gut microbiome includes gut microbiota composition (i.e., bacteria) and function (i.e., SCFAs). For appetite regulation, studies reporting appetite ratings and/or hormones (ghrelin, PYY, and GLP-1) were reviewed. Lastly, studies measuring markers of adipose or systemic inflammation were included in the inflammation section of this review. The final section of this review article provides concluding remarks and suggested future directions.

This thesis project is in the form of a narrative literature review on the effect of common bean consumption on the gut microbiome, appetite regulation, and inflammation in obesity. The final chapter of this thesis summarizes the findings of the review articles, presents strengths and weaknesses of the review, and highlights research gaps to direct future studies.

Research problem:

Research has shown NDCs and polyphenols have the ability to influence the gut microbiota, appetite regulation, and inflammation[8,9]. Although dietary pulses are a food high in both NDCs and polyphenols, research on the ability of pulses to modulate these factors is less abundant. More specifically, knowledge on the impact of common beans on the gut microbiome, appetite regulation, and inflammation, especially in individuals LWO, is limited and has yet to be summarized in a cohesive manner.

Objective:

The objective of this thesis was to summarize the current evidence on the impact of common beans on gut microbiota, appetite regulation, and inflammation in animal models of obesity and humans LWO, and to discuss areas that require further research in hopes of highlighting the potential for beans as a therapeutic avenue for the management of obesity, as well as to highlight the need for controlled studies in this research area.

Chapter 2: Methodology

No strict protocol in terms of methodology was used due to the narrative nature of the review article. This section presents a general description of the methods used to search the literature related to the topic of the review.

Articles were searched in the following databases: PubMed, Scopus, and Medline. Articles were included if they were written in English and published in peer-reviewed journals. There were no restrictions on publication date. Main search terms were gut microbiota/microbiome/bacteria; obesity; dietary pulses; common beans; Phaseolus vulgaris; appetite (hormones, ratings, sensations); inflammation. There were no restrictions on samples size, participant age or sex, pulse dose, or study duration. Both human and animal models were included. Retrieved articles were selected if they met the inclusion criteria after reading the full article. Reference lists from relevant publications were manually searched for any articles missed by the original search.

Inclusion/Exclusion Criteria

Inclusion criteria: participants LWO or animal models of obesity, whole pulses, single meal or long-term consumption, and measured at least one of the variables of interest. Variables of interest were inflammatory markers (EAT or blood), appetite control (hormones or ratings), gut microbiota composition (fecal or cecal) and/or function (SCFA concentrations). Interventions with multiple pulse varieties were included if common beans were used and studies using pulse fractions were excluded. The inclusion criteria for participants LWO was removed if no such studies could be identified.

Chapter 3: Review Article

This review article has been submitted for publication and is currently *"Under Review"* in *Obesity Reviews* (OBR-07-22-5807; submitted July 18 2022).

Title

Dietary pulses as an accessible means to improve the gut microbiome, inflammation and appetite control in individuals with obesity

Abstract

Interest in the gut bacterial community residing in the human intestine, otherwise known as the gut microbiota, has exploded in recent years. The gut microbiome has been linked to chronic diseases such as obesity, suggesting interventions that target the microbiome may be useful in treating obesity and its complications. Dietary pulses (*e.g.*, common beans) are composed of nutrients and compounds that possess the potential to modulate gut bacteria composition and function which can in turn improve appetite regulation and chronic inflammation in obesity. This review summarizes the current state of knowledge regarding the connection between the gut microbiome and obesity, appetite regulation, and systemic and adipose tissue inflammation. More specifically, it highlights the efficacy of interventions employing dietary common beans as a means to improve appetite regulation and inflammation in both rodent obesity and in humans. Collectively, results presented and discussed herein provide insight on the gaps in knowledge necessary for a comprehensive understanding of the potential of beans as a treatment for obesity while highlighting what further research is required to gain this understanding.

Keywords: Gut microbiota, appetite, inflammation, dietary pulses.

1.0 Introduction

According to the World Health Organization, the prevalence of obesity worldwide has nearly tripled between 1975 and 2016, and over 650 million adults are living with obesity (LWO) [1]. Obesity is associated with several health conditions, such as cardiovascular disease and chronic systemic low-grade inflammation [2], and puts financial strain on healthcare systems around the world [3]. It has been shown that improvements in health markers (e.g., pro-inflammatory markers) and reductions in mortality risk can occur in the absence of weight loss [4]. Additionally, it has been found that on average, individuals that have lost weight will regain a third of it back within a year, and will go on to regain the remainder of the weight back within 3-5 years [5]. Current obesity interventions, such as calorie-restriction diets, pharmacotherapy, and bariatric surgery [6], target weight loss despite such a high likelihood of weight regain later on. It has recently been recommended that the first step of obesity management be the prevention of further weight gain [7]. Furthermore, current treatments for obesity have significant barriers to accessibility, e.g., cost, time, and availability [8]. There is a need for research and development of obesity treatments that are focused on preventing further weight gain and improving health outcomes while removing as many accessibility barriers as possible.

The gut microbiome has been a topic of great interest in recent years. The gut microbiome exists within the gastrointestinal tract and is composed mainly of bacteria (i.e., microbiota or microbes), followed by fungi, viruses, and archea, that contribute to metabolic functions such as immune function and nutrient digestion and absorption [9]. In addition to numerous metabolites, the gut bacteria ferment non-digestible carbohydrates (NDCs) and polyphenols which results in the production of short-chain fatty acids (SCFAs) and phenolic compounds. These metabolites have

been shown to interact with processes responsible for appetite regulation and systemic and adipose tissue inflammation [10,11]. A treatment that targets the gut microbiota in a way that improves microbiota composition and facilitates the production and utilization of metabolites produced via fermentation of NDCs and polyphenols may improve appetite regulation and inflammation in individuals LWO. Dietary pulses are a relatively inexpensive high-protein food source that has the potential as a dietary intervention that targets the gut microbiota due to their high NDC and polyphenol content [12].

As will be discussed in this review, studies have shown the potential for dietary pulses, more specifically common beans, to modulate the composition and function of the gut microbiota, regulate appetite, and attenuate inflammation. However, there are few, if any, comprehensive studies that measure all of the aforementioned variables in response to a common bean dietary intervention in the context of obesity. The aim of this review is to summarize the current evidence on the impact of common beans on gut microbiota, appetite regulation, and inflammation in human and animal models of obesity, and to discuss areas that require further research.

2.0 What are Dietary Pulses?

Dietary pulses are non-oilseed legumes and are described as the dried, edible seeds of grain legumes [12]. Types of pulses include beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), and dry peas (*Pisum sativum*). Pulses are a nutrient dense food that are a rich source of NDCs, polyphenols, and plant proteins. NDCs escape digestion in the human small intestine and can be generally classified as insoluble (e.g. cellulose) or soluble fiber (e.g. oligosaccharides) [13]. After escaping digestion in the upper gut, some NDCs are fermented by gut

bacteria in the ileum and colon [14], resulting in the production of SCFAs. There is significant variation in the quantity of total dietary fiber, insoluble fiber, and soluble fiber in each pulse type. Common beans have higher total dietary fiber (23-32g/100g) compared to chickpeas, lentils, and dry peas (range of 18-26g/100g) [15]. Polyphenols are bioactive phytochemicals with many metabolic fates, one being the catabolism by colonic gut bacteria resulting in the production of metabolites such as phenolic acids (e.g. benzoic acids)[16]. They are mainly found in the seed coat of beans, and the polyphenol content differs between pulse types and varieties based on seed coat color [17]. This review will focus on dietary interventions utilizing common beans due to their relatively high fiber and polyphenol content compared to other pulse types.

3.0 Common Beans Effects on the Microbiota in Obesity

3.1 Common Beans Modulate Gut Microbiota Composition and Function

One way in which common beans have the ability to modulate the gut microbiota is through their high content of NDCs and polyphenols. NDCs are an energy source for the microbiota, promoting the growth of bacteria thereby acting as a prebiotic [18]. Prebiotics can be defined as "selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota" [19]. NDCs can be fermented by gut bacteria in the ileum and colon after having escaped digestion in the upper gut [20]. This fermentation not only results in a shift in the composition of the microbial community, but also in the production of several end products, such as SCFAs [20]. The level of fermentability varies between types of NDCs. For example, pectin, β-glucan, and resistant starches are highly fermentable while cellulose is poorly fermentable [21]. Fermentability directly correlates to SCFA production; highly fermentable fibers produce higher amounts of SCFAs and vice versa [22]. Similarly, polyphenols have a bidirectional relationship with

the gut microbiota. On the one hand, polyphenols are metabolized by the gut microbiota which enhances their bioavailability and bioactivity, compared to their parent plant compounds [23]. Among many physiological effects of polyphenols, metabolites produced from the metabolism of polyphenols by the gut microbiota have been shown to have systemic anti-inflammatory properties and reinforce the gut barrier [24]. On the other hand, polyphenols have also been shown to modulate the composition of the microbiota, being recognized as potential prebiotic compounds [16]. The complex mechanisms of action for interactions between polyphenols and gut microbiota are outside the scope of this review and have been previously reviewed elsewhere [16,20].

3.2 Dysbiosis in Obesity

In absence of inflammation and disease, microbes and their host have a mutually beneficial symbiotic relationship. A disruption in the microbial community can result in dysbiosis, which can be defined as "any change in the composition of resident commensal communities relative to the community found in healthy individuals" [25]; resident commensal communities referring to gut microbiota composition. Previous studies have identified that dysbiosis in human and rodent models may contribute to diseases such as inflammatory bowel disease, diabetes, and obesity [25]. However, it is unknown whether these observed changes in microbial community structure are a contributing factor or a result of the disease itself.

Dysbiosis has been documented in both humans and mouse models of obesity, although current research has considerable variation in reports of the specific microbial community structure characteristic of obesity. A systematic review from 2020 evaluated 32 observational human studies and clinical trials that investigated and compared the gut microbiota composition of adults LWO

and of normal weight [26]. Three of the 32 studies found no differences between the gut microbiota of individuals LWO and those of normal weight. Results from the remaining studies demonstrated some microbes that appeared characteristic of obesity, although not all studies were in agreeance. Microbial diversity within samples, referred to as α-diversity, was reported by only 4 studies. Lower α-diversity was characteristic of obesity in 3 studies, with only 1 study reporting higher α-diversity in obesity.

Changes in microbial diversity can often be characterized by changes in abundance of phyla, families, genera, and species. In Crovesy *et al.*'s systematic review (2020) [26], abundance of the phylum Firmicutes was commonly reported as higher in obesity than normal weight, although 2 studies reported a lower abundance. Firmicutes have previously been linked to obesity due to an increased capacity for energy harvest [27]. Members of this phylum have more carbohydrate metabolism enzymes, allowing for increase capacity to metabolize otherwise indigestible carbohydrates [27]. This review found a lack of consensus on the relative abundance of Bacteroidetes in obesity. Out of the studies that reported the abundance of Bacteroidetes, 6 studies reported lower and 5 reported higher relative abundance in individuals LWO. The Bacteroidetes phylum has been shown to have a positive correlation with body fat reduction [28]. Some studies also calculated a Firmicutes/Bacteroidetes (F/B) ratio; out of these studies, with the majority reporting a higher ratio in obesity [26]. One study has reported that in obesity, an increased F/B ratio is particularly characteristic of individuals with a body mass index (BMI) > 35 kg/m^2 [29]. Conversely, Schwiertz *et al.* (2010) [30], who reported a lower ratio of F/B, found the ratio to be over 50% lower in individuals LWO. Individuals of normal weight had a F/B ratio of 3.3 and subjects LWO had a ratio of 1.2 [30]. Mouse models have been more consistent in showing an increase in Firmicutes and

decrease in Bacteroidetes abundance in *Ob/ob* mice [31] and high-fat diet (HFD)-induced obesity mouse models [32]. Proteobacteria and Actinobacteria are two phyla that are also found to be reduced in both human and mouse models of obesity [33,34].

Multiple families, genera, and species have been identified in the microbiome of individuals LWO. For example, the *Lactobacillus* genus belongs to the Firmicutes phylum and has been linked with obesity. *L. reuteri* has been positively correlated with BMI [35]. Interestingly, other bacteria from this genus have found to be reduced in obesity. It has been reported that *L. paracasei* and *plantarum* have a protective effect against weight gain and are reduced in obesity [26]. These bacteria produce antimicrobial peptides that are involved in innate immune responses and prevent the growth of bacterial pathogens that contribute to dysbiosis [36]. *Prevotella*, a SCFA-producing bacteria, has been shown to be reduced in obesity [26] and negatively correlated with BMI [37]. However, Schwiertz *et al*. (2010) [30] reported no significant differences in *Prevotella* abundance in individuals of normal weight compared to those with overweight and obesity. Both *Ruminococcus flavefaciens* and *R. gnavus* have been reported as reduced in obesity [30,38]. These bacteria aid in the degradation and fermentation of resistant starches [39]. *Akkermansia muciniphila*, a member of the Verrucomicrobia phylum, is found to be reduced in obesity in both mice and humans [40,41]. This microbe has been shown to increase fecal energy content by reducing the absorption of carbohydrates in the jejunum [41,42], reduce intestinal barrier permeability [43], and has shown to be negatively associated with BMI, waist-to-hip ratio, and adipocyte diameter in humans [44]. In summary, the presence of microbial dysbiosis has consistently been shown in human and mouse models of obesity, and although the relative abundance of specific microbes in obesity has yet to

be confirmed, it is possible that interventions which modulate the microbiota composition in obesity may have beneficial downstream health impacts.

3.3 Common Beans as a Treatment for Obesity-Related Dysbiosis: Animal Trials

Research has consistently demonstrated the ability of common beans to alter the gut microbiota community structure in mouse models during the development of HFD-induced obesity (See **Table 1**). When compared to a HFD control group, the addition of common beans to a HFD has significantly increased α-diversity [45–47]. The abundance of *A. muciniphila* has been increased 2.1, 19-, and 473-fold when different common bean varieties were added to a purified HFD for 12-17 weeks [46,45,48]. These results are in comparison to purified HFD controls. Common beans have frequently elicited a reduction in the F/B ratio [45,48,49], although no significant change in this ratio has also been reported [46]. Interestingly, Tan *et al.* (2021) [49] reported a ratio that was 64.1% lower in bean-fed male C57BL/6J mice compared to a control group, despite no significant change in abundance of Firmicutes. Actinobacteria abundance has been reported as both decreased [46] and unchanged [45] in response to bean supplementation. Bean consumption has also been found to increase [46,47] and decrease [45] abundance of Proteobacteria. *R. gnavus* was decreased 1.5-fold in male C57BL/6 mice following 12 weeks of navy bean consumption compared to a control [46]. Common bean consumption for 17-weeks has also successfully reduced *R. gnavus* abundance in male C57BL/6NCr1 mice [45]. Other bacteria commonly reduced in obesity, *Prevotella* and S24-7, have increased in response to pulse consumption [45–47,49]. In one study, navy bean supplementation for 12-weeks increased *Prevotella* abundance by 332-fold in male C57BL/6 mice compared to a control group [46].

It is also important to consider the ability of common beans to modulate the gut microbiota after obesity has been established in mouse models because the existence of microbial dysbiosis prior to bean consumption is more representative of human interventions. Additionally, the magnitude of effect of different pulse types should be considered when multiple types are used within a dietary intervention due to varying NDC and polyphenol content in pulses. After establishing HFD-induced obesity, mice either continued consuming the HFD alone or began consuming common beans in addition to the HFD[50–52]. α-diversity was found to be both increased [50] and decreased [51] in response to 12-weeks of white kidney bean consumption and 8-weeks of navy bean consumption, respectively. Compared to those consuming a HFD alone, mice fed bean supplemented HFDs had increased the abundance of *A. muciniphila*, *Prevotella*, S24-7, and Bacteroidetes, and decreased the abundance of Firmicutes as well as a decreased F/B ratio [50–52]. These results are consistent with studies showing modulation of the mouse gut microbiota during the development of HFD-induced obesity, suggesting beans may elicit similar effects when consumed during the development of obesity compared to consumption in established obesity. Additionally, when compared to the same dose of chickpeas and dry peas, 40% (wt/wt) *Phaseolus vulgaris* elicited a greater effect on gut microbiota composition of male NCI C57BL/6NCr mice after 12 weeks of consumption [50]. When looking at *A. muciniphila* abundance, common bean-fed mice had a 49.4-fold increase while lentil-fed mice had a 24.6-fold increase [50].

SCFA concentrations are indicative of gut microbiota function and were only measured in three of the studies discussed so far in this section [46,47,51]. Total fecal SCFA concentrations were increased in male C57BL/6 mice fed a purified HFD (59% by kcal) to induce obesity supplemented with 15.7% (wt/wt) navy bean powder for 8 weeks [46]. This increase in SCFAs was accompanied by

increases in SCFA-producing bacteria *Prevotella* and S24-7. Increases in fecal SCFA concentrations in response to common beans has also been observed in mice with established obesity. Male C57BL/6 mice with established obesity had increases in total and individual (acetic, propionic, and butyric acid) fecal SCFA concentrations in response to 8-weeks consumption of a purified HFD (59% by kcal) supplemented with 15.7% (wt/wt) navy bean powder compared to high-fat and low-fat controls [51]. Conversely, common beans have been reported to decrease total fecal SCFA concentrations during diet-induced obesity of male Wistar rats [47]. The consumption of 79.8% (wt/wt) dry cooked black beans in addition to a purified HFD for 2 months in male Wistar rats reduced total fecal SCFA concentrations, but increased fecal butyrate concentrations, compared to a control diet [47]. The fat content of the HFD was not reported. This study design, particularly the dietary intervention, has been critiqued for differences in the composition of diets between groups [53]. For example, the high-fat control diet and the HFD supplemented with black beans contained 7.8g and 0g of sucrose (per 100g), respectively. The differences in sucrose between diets was critiqued due to the possibility of consequential changes to intestinal microbiota from high sucrose levels in the diet [53,54].

It must be noted that to measure gut microbiota composition and function, some studies use DNA from cecal contents collected at necropsy, while others extract DNA from fecal samples. Diversity and composition of the microbiome can differ depending on the location of the gastrointestinal tract [55], meaning location of the microbiota samples should be taken into consideration when comparing results. As shown in **Table 1**, there was no consistent location for gut microbiota measurements within the animal studies. In humans, however, measurements are mostly taken from the feces due to the inability to easily obtain microbial data from the human cecum. SCFA

concentrations in the blood would allow for a measure of absorbed SCFAs but were not taken in these studies. Inconsistencies between studies discussed in this section may be explained by factors such as bean type and quantity. For example, black bean powder supplementation did not alter α-diversity when added to a purified HFD [49], but supplementation of a HFD with other common beans, such as navy beans, increased α-diversity [46]. Polyphenol content varies between common bean varieties based on seed coat colour [17], meaning it is possible that bean varieties may have differing effects on the microbiota. Many studies did not clarify the common bean type used leading to difficulty comparing results [45,50,52]. Bean dosage varied greatly between studies, ranging from 15.7 to 79.8% (wt/wt) which may have influenced results. A limitation consistently seen across these studies is the exclusion of mice of the female sex. Only recently has the relationship between bean dosage, sex, and gut microbiota composition been documented[56].

To investigate the influence bean dosage and sex have on the gut microbiota, Lutsiv *et al.* (2022)[56] used 20-day-old male and female C57BL6/J mice with an established obese phenotype and fed them one of four diets for 12-14 weeks, after which bacterial measurements were taken from the cecum. Prior to bean supplementation, the mice were fed an obesity-inducing purified diet (32.5% fat by kcal) for 8 weeks. From least to greatest dose, white kidney bean powder was added to the purified diet by substituting casein with 0%, 17.5%, 35%, and 70% of the protein derived from bean. In other words, these diets had 0, 10.2, 20.4, and 40.8g white kidney bean/100g total diet, respectively. Multiple differences in microbial populations were found between doses and sexes and are detailed in **Table 1**. The magnitude of phylum-level changes in both sexes increased as bean dose increased. The F/B ratio decreased with increasing bean dose in both female (range 0.421-0.232) and male mice (range 0.528-0.360). Additionally, the decrease in Firmicutes

abundance was much larger in female than male mice, ranging from 19.18-25.8% and 10.14-13.41%, respectively. Female mice continued to see a greater effect of bean treatment on phylum abundance compared to male mice, as shown by a larger decrease of Actinobacteria and increase of Bacteroidetes abundance. Interestingly, Verrucomicrobia was significantly reduced only by the 70% bean diet in both sexes. There were some sex-specific alterations in the gut microbiota, e.g., suppression of *A. muciniphila* exclusively in female mice on the 70% bean diet and enhanced *Sutterella* exclusively in female mice fed the 35% and 70% bean diets. In addition to confirming the ability of common beans to modulate the gut microbiota in obesity, this study highlights the importance for acknowledging and accounting for dose- and sex-specific responses to pulse treatments during analysis.

Overall, studies on bean-induced alterations in gut microbiota composition in obesity report fairly consistent evidence supporting the idea that common beans can attenuate obesity-associated dysbiosis, as listed in **Table 1**. An example of this would be the increase in Bacteroidetes abundance and decrease in Firmicutes abundance (See **Table 1**). Although few studies measured gut microbiota function, results have shown that navy beans increase total concentrations of fecal SCFAs both during and after diet-induced obesity [46,51]; however, black beans decreased total fecal SCFAs, although this may have been due to previously mentioned issues with the study design [47]. Multiple studies did not document the variety of common beans used; future studies should include this information as bean varieties can vary in nutritional composition [17]. Future studies should further examine the impact of common beans on gut microbiota function (i.e., SCFAs) and further explore the role of sex in the modulation of gut microbiota composition and function.

3.4 Common Beans as a Treatment for Obesity-Related Dysbiosis: Human Trials

There are few studies that have been conducted to investigate the effects of pulse consumption on the gut microbiota in individuals LWO, especially those using common beans (*Phaseolus vulgaris*) as the dietary intervention. A systematic review published in 2020 searched for studies that looked at changes in human gut microbiota in response to dietary pulse consumption; 2444 studies were identified, and only five randomized-controlled trials fit the search criteria [57]. The duration of the studies analyzed varied from 3-12 weeks and pulse dose varied from 35g/day (powder weight) to as much as 200g/day (dried cooked weight). Pulse Canada has previously published an article recommending 100g of cooked pulses daily [58]. The systematic review did not find a consistent effect of pulses on the gut microbiota, noting a main limitation being a large variation in methodologies between studies. Out of the five studies analyzed, two used common beans and included individuals overweight or LWO[59,60]. These two studies are discussed in further detail below.

The first of the two aforementioned studies measured differences in the abundance of 6 carbohydrate-fermenting bacterial species (*Bifidobacterium longum, Bacteroides vulgatus, Clostridium clostridiiforme, Methanobrevibacter smithii, Peptostreptococcus productus,* and *Eubacterium limosum*) by PCR, in men and women with and without pre-metabolic syndrome (n=10/group) before and after the consumption of 130g canned pinto beans/day or a placebo (chicken noodle soup) for 12 weeks [59]. The main criterion for pre-metabolic syndrome was defined as having a waist circumference ≥96.5 cm for men and ≥88.9 cm for women. Participants with pre-metabolic syndrome also had obesity (BMI ≥30 kg/m^2). While there were no changes in the abundance of 5/6 bacterial species measured between groups, it was found that *Eubacterium*

limosum abundance, a SCFA-producing bacteria, was significantly lowered by bean consumption in both individuals with and without pre-metabolic syndrome. Furthermore, as a measurement of microbial activity, an aliquot of the collected fecal samples were used in an *in vitro* fermentation experiment to determine the potential for the bean-fed microbiota to produce SCFAs following incubation with various substrates (dried bean powder, cornstarch, inulin, and oat bran). The results showed that the total SCFAs and propionate were significantly increased in the feces from the bean group, only when dried bean powder was used as the substrate. A limitation of this study was that only 6 bacterial species were isolated during analysis, most likely due to restraints on cost, time, and resources. Targeting specific bacteria during analysis only provides insight on a small portion of the gut microbiota, leaving bacterial species that may have been affected by the bean treatment unaccounted for. Additionally, SCFA concentrations were analyzed using *in vitro* fermentation as opposed to directly in the samples collected which may have altered the original microbial community and its ability to ferment substrates. Although limited, the results show that daily consumption of pinto beans results in a change in the structure (i.e., increased abundance of *Eubacterium limosum*) and function (i.e., increased total fecal SCFAs and propionate) of the microbial community.

In the second two aforementioned bean-intervention studies reviewed by Marinangeli *et al.* (2020)[57], navy beans were shown to alter gut microbiota populations in male and female colorectal cancer survivors with overweight or obesity [60]. α-diversity was significantly increased after 4 weeks of daily consumption of foods cooked with navy bean powder (35g/day). After just 2 weeks of navy bean supplementation, the relative abundance of *Bacteroides fragilis* was decreased and *Lachnobacterium spp.* was increased. *B. fragilis* remained significantly decreased after 4 weeks of

bean consumption, in addition to decreased mean abundance of an unclassified *Clostridium* and *Anaerostipes* and increased unclassified *Lachnospira* and *Coprococcus*. The bean diet did not produce any phylum-level changes in the gut microbiota or changes in fecal SCFA concentrations. This study not only demonstrates the ability of navy beans to alter gut microbiota composition in overweight/obesity, but also the timeline in which these changes can occur. Although the average BMI of participants was overweight (27.3 ± 3.3 control, 28.5 ± 7.9 bean diet), BMI ranged from 18-46.4 kg/m^2 and 21 of the 30 participants had BMI's classified as overweight or obese. No separate analysis was performed to determine the contribution of BMI to the response of the microbiota to the pulse intervention. In summary, pinto and navy beans have successfully altered microbial community structure in individuals LWO, although there are conflicting results on the effect of beans on gut microbiota function in individuals LWO. Due to the lack of studies on this topic, further studies should first investigate and detail specific alterations in the microbiome induced by common bean consumption in individuals LWO before determining the influence of other factors such as dosage and sex.

4.0 Common Bean Effects on Appetite Regulation in Obesity

4.1 Gut Peptides and Appetite Regulation

Appetite is one of multiple factors that regulates energy intake and is related to several episodic and tonic hormonal signals. Episodic signals are responsive to episodes of feeding while tonic signals are involved in long-term regulation of appetite and satiety, mostly in relation to fluctuations in energy stores; *s*atiety defined as the feeling of fullness and suppression of hunger following a meal [61]. Appetite sensations (i.e., ratings of hunger, fullness, desire to eat, and prospective food consumption) reflect subjective appetite and are a good predictor of short-term

energy intake [62]. Gut hormones secreted by enteroendocrine cells influence appetite and can stimulate (orexigenic) or suppress (anorexigenic) appetite. Among these hormones, gut peptides glucagon-like peptide *(*GLP)-1 and peptide YY (PYY) have been shown to slow the rate of gastric emptying and intestinal transit time; physiological processes that increase satiety [63]. Furthermore, these hormones regulate appetite by inhibiting and/or stimulating neuropeptides in the arcuate nucleus of the hypothalamus. These neuropeptides can be orexigenic, such as neuropeptide Y (NPY) and agouti-related peptide (AgRP), or anorexigenic, such as proopiomelanocortin (POMC). Both GLP-1 and PYY are secreted by the L-cells of the distal ileum and colon. The satiating effects of GLP-1 and PYY are brought by inhibiting NPY/AgRP neurons and promoting POMC neurons in the hypothalamus [64]. Ghrelin is the only gut peptide known to have an orexigenic effect. This gut hormone is almost entirely secreted from the stomach and increases appetite through the stimulation of NPY/AgRP neurons and inhibiting POMC neurons [64].

4.2 Appetite Regulation in Obesity

Under "normal" circumstances, GLP-1 and PYY are secreted in response to food consumption in proportion to caloric content of the food [64]. Studies have reported decreased postprandial concentrations of PYY and GLP-1 in individuals LWO compared to normal-weight individuals [63]. The postprandial PYY response has been absent in class-II (BMI 35.0–39.9 kg/m^2) and -III (BMI≥40 kg/m^2) obesity [65]. GLP-1 has been shown to peak early and at lower levels postprandially in obesity, with class-III obesity having no postprandial GLP-1 response at all [65]. Diet-induced weight loss can further exacerbate any dysregulation of PYY and GLP-1 secretion in both fasted and postprandial states, and these alterations have shown to persist 1-year after weight loss in individuals that have maintained weight loss and in those that have gained a portion of the weight

back [66]. Ghrelin is secreted during the fasting state and decreases following food consumption proportional to the caloric content [67]. Individuals LWO may experience less suppression of ghrelin postprandially [67], and the postprandial inhibition of ghrelin can be completely blunted in individuals with class-III obesity [65]. Furthermore, weight loss is accompanied by an increase in both fasting and postprandial ghrelin concentrations [67]. In terms of appetite sensations, increases in desire to eat and hunger as well as a decrease in fullness have been reported following weight loss [68]. As such, identifying strategies that in turn normalize gut peptide profiles in individuals LWO before and after weight loss, could beneficially impact appetite regulation and may also improve body weight control.

4.3 The Effects of Common Bean Consumption on Appetite Hormones in Rodent Models of Obesity

HFD-induced obesity mouse models can provide mechanistic insight on the ability of common beans to attenuate obesity-related alterations in appetite gut hormones. Appetite hormones GLP-1 and ghrelin were measured in a fasted state following dietary supplementation of 20% black beans in male mice in which diet-induced obesity [49]. In this study, male C57BL/6J mice consuming a black bean diet were fed a 46% (by kcal) purified HFD in addition to 20% (wt/wt) bean powder made from cooked and freeze-dried black beans, and the control groups were fed a high-fat or low-fat purified diet, all for a duration of 6 weeks. This study found that serum levels of ghrelin were unchanged by the bean diet, while GLP-1 was significantly reduced following bean consumption. The bean diet increased fasted GLP-1 by 21.6% compared to the HFD and to a concentration similar to that of the low-fat control [49]. Although GLP-1, an anorectic hormone, was reduced by the bean diet, total energy intake was significantly lower in bean-fed mice compared to the HFD

control group. Body weight was not significantly different between the bean-diet and HFD control group. On the contrary, supplementation of a HFD with 15.7% (wt/wt) navy beans in male mice with established obesity produced changes in fasted serum levels of ghrelin but not GLP-1 [51]. In this study, obesity was first established in male C57BL/6 mice by consumption of a 59% (by kcal) purified HFD for 12 weeks after which mice either continued on a HFD (obesity control), were fed a HFD supplemented with 15.7% cooked navy bean powder, or were fed a low-fat diet (LFD) (weight-loss control) for an additional 8 weeks [51]. Compared to the HFD and LFD controls, mice fed bean powder had significantly reduced fasted serum levels of ghrelin, while GLP-1 remained unchanged in all groups. The LFD produced a significant reduction in body weight compared to the HFD control and HFD bean supplemented groups, whose body weight did not differ.

One strength of using animal models for appetite regulation studies is the accuracy they provide for measurements of energy intake due to the ability to completely control the environment. Another strength of the studies discussed above [49,51] was the prescription of bean supplements in quantities equivalent to realistic daily intake of beans in humans (e.g., 15.7% (wt/wt) is equal to ~1 cup/day) which allows for better comparison and translation between animal and human interventions. In summary, the very limited number of studies using mouse models that employed common beans as an intervention for preventing or treating obesity-related appetite hormone dysregulation have showcased the ability of black and navy beans to modulate appetite hormones GLP-1 and ghrelin, although results were not consistent. More studies are needed to consolidate the direction and magnitude of the effects on fasted concentrations of appetite hormones GLP-1, PYY, and ghrelin, and perhaps investigate postprandial effects as well.

4.4 The Effects of a Single Bean Meal on Appetite and Energy Intake: Human Trials

To the best of our knowledge, there are no studies to date that investigate the effect of a pulse meal on appetite regulation exclusively in individuals LWO; however, this topic has been well documented with normal weight populations. Therefore, to provide insight on the appetite-regulating effects of a single pulse meal, this section will discuss trials that include participants without obesity. A 2014 systematic review found that in normal weight individuals, whole pulse meals have been found to increase satiety, as measured by visual analog scales (VAS), but had no effect on energy intake at a second meal [69]. Pulse types included in this review were lupin, lentils, chickpeas, yellow peas, and common beans and pulse meal size varied greatly from 7.6-311g/pulses per meal. This analysis included 9 trials with a median BMI of 24.7 kg/m^2. There was an average increase of 31% in postprandial satiety following pulse meals when compared to control meals [69]. The length of time for which postprandial measurements were taken varied from 120-270 minutes. Furthermore, 7 trials included food intake at a second meal that followed the whole pulse meal, but there was no significant effect of dietary pulses on second meal food intake despite an overall increase in satiety. Gut peptides involved in appetite regulation were not measured in these studies.

In one study, the impact of pulse dose on appetite and energy intake was assessed in participants with a BMI range of 17.9-33.2 kg/m^2 (average BMI 24.8 ± 1.3 kg/m^2) [70]. The purpose of this study was to compare the effect of a meal containing a low dose of dietary pulses (1/2 cup) versus a high dose (1 cup) on satiety. Results showed no significant change in satiety scores 120 minutes after consuming meals with either a low or high dose of pinto or navy beans [70]. Energy intake was measured after the test meals for the remainder of the day; both calorie and macronutrient

composition were not significantly affected by any test meal. This study did not measure gut hormones involved in appetite regulation. Although this study included individuals LWO, no analysis was done to control for BMI. Therefore, results from this study should be interpreted accordingly.

Appetite hormones in normal weight adults following the consumption of a bean meal have also been documented. Brown beans or white wheat bread were consumed as an evening meal and PYY, GLP-1, ghrelin, and appetite ratings were measured the following day in a fasted state (11-14 hours after meal consumption) as well as 0-180 minutes after a subsequent standardized breakfast [71]. Although GLP-1 concentrations were unchanged, participants had 51% higher levels of PYY in both the fasted state and 0-180 minutes postprandially, and ghrelin concentrations were 14% lower 0-180 minutes postprandially, after consuming the brown bean meal compared to the white wheat bread evening meal. Hunger sensations were 15% lower following the brown beans, but only for 0-45 minutes post-breakfast. Willingness to eat at 0-45 minutes postprandial was inversely related to plasma concentrations of PYY. Although studies with normal weight participants suggest a single pulse meal has appetite-suppressing effects, the potential of a bean meal on appetite regulation in individuals LWO will not be fully understood until studies are dedicated specifically to this population and both appetite hormones and sensations are measured.

4.5 The Effects of Long-Term Bean Consumption on Appetite and Energy Intake: Human Trials

Similar to acute appetite trials, there are limited long-term appetite studies prescribing a common bean diet to individuals LWO. The following trial includes participants LWO (average BMI 32.8±0.7 kg/m^2) and prescribed a dietary intervention that included a variety of pulse types, as

opposed to exclusively common beans. Mollard *et al.* (2012)[72] conducted an 8-week study wherein individuals with overweight and obesity were assigned to one of two diet groups: a calorie-restricted diet (-500 kcal/day) or habitual diet supplemented with a variety of pulse types. Those in the pulse group were prescribed an average of 896g pulses/week in the form of pre-made meals containing lentils, chickpeas, yellow peas, and navy beans. Fasted and postprandial concentrations of GLP-1 and ghrelin were measured at baseline, week 4, and at the end of the intervention. Fasted serum concentrations of ghrelin and GLP-1 did not change compared to baseline values in either diet group at any time point. Blood was also collected at 30, 60, 90, and 120 minutes following a 75g glucose oral solution, but there was no effect of diet on ghrelin or GLP-1. The effect of common beans specifically on appetite hormones cannot be isolated in this study due to the mixed pulse design of the dietary intervention. Although the pulse diet was designed to be energy balanced, 24-hour dietary recalls revealed that daily energy intake was significantly reduced in the pulse group to values close to that of the calorie-restricted group. The authors of this study attributed the reduction in caloric intake to the incorporation of "higher energy-density and carbohydrate foods" in the diet [72]. Ratings of appetite sensations may have provided an additional explanation for the reduced energy intake in the pulse diet group, but no appetite sensations were measured in this study.

In summary, studies that investigate short-term appetite regulating abilities of a single pulse meal have found increased satiety in individuals with normal weight [69], no change in satiety in a sample of participants with great variation in BMI [70], and increased PYY, unchanged GLP-1, and reduced ghrelin in individuals with normal weight [71]. Additionally, an 8-week mixed pulse dietary intervention reduced energy intake but did not alter appetite hormones or in individuals LWO [72].

Body weight remained stable during this intervention. Overall, there is need for studies that measure short- and long-term regulation of both appetite hormones and sensations in individuals LWO as a result of common bean consumption. To the best of our knowledge, there are no studies on this topic that 1) recruit participants with obesity and not normal weight, 2) have a dietary intervention that exclusively includes common beans, and 3) measures appetite hormones and ratings. A consistent effect of common beans on appetite regulation in obesity cannot be determined until further research is completed.

4.6 Is the Effect of Beans on Appetite Regulation Mediated Through Their Effects on the Microbiota?

One component of beans which may alter appetite regulation is dietary fiber which is well known for its appetite-suppressing abilities, as reviewed by [73]. In addition to reducing the energy density of foods, increasing mastication time, and decreasing intestinal transit time [73], dietary fiber can influence appetite via the production of SCFAs. SCFAs are microbial metabolites produced during the fermentation of NDCs by gut bacteria and play a role in appetite regulation via increasing the secretion of anorectic gut hormones PYY and GLP-1 and inhibiting the secretion of orexigenic gut hormone ghrelin; the appetite-regulating role of SCFAs has been previously reviewed by Byrne *et al.* [74]. SCFAs activate G-protein-coupled receptors (GPRs) which stimulate enteroendocrine L-cells to secrete, or inhibit, gut hormones such as PYY and GLP-1, and ghrelin, respectively [75(p41)]. Receptors specifically linked to these gut hormones are GPR41 and GPR43, both of which have differing affinities for the three primary SCFAs produced during fermentation (acetate, propionate, and butyrate); GPR43 has equal affinity for acetate, propionate, and butyrate, and GPR41 has greater affinity for propionate and butyrate compared to acetate [76]. The binding of SCFAs to

GPR43 stimulates the secretion of GLP-1 and PYY [10] and inhibits the secretion of ghrelin [77], and binding of SCFAs to GPR41 also stimulates the secretion of PYY [78].

In connection with this, the abundance of certain bacterial groups have been linked to concentrations of gut peptides GLP-1, PYY, and ghrelin [79–81]. For example, as recently reviewed by Leeuwendaal *et al.* [80], the abundance of *Clostridium*, *Ruminococcus,* and *Bacteroides* was found to be positively correlated with ghrelin concentrations, while a negative association was found between Bacteroidetes/Firmicutes ratio and ghrelin. PYY and GLP-1 have also been linked to gut bacteria. Mice fed a HFD, in addition to the administration of *A. muciniphila* for 12 weeks had significantly increased PYY and GLP-1 mRNA expression [81]. This was accompanied by a significant decrease in caloric intake in these models. *Faecalibacterium prausnitzii, Bifidobacterium,* and *L. reuteri* have also been associated with increased GLP-1 secretion [79]. It is likely that the SCFA-producing abilities of these bacteria links them to gut peptides such as ghrelin, PYY, and GLP-1 [20]. The main SCFA-producing bacteria belong mostly to Firmicutes, Actinobacteria, and Verrucomicrobia phyla [82]. These species include, but are not limited to, *F. prausnitzii, Eubacterium rectale, Bifidobacterium,* and *A. muciniphila* [82]. Therefore, since the consumption of common beans can modulate the composition of the microbiota and enhance the production of SCFAs, regulation of appetite control through the gut microbiota may be an important target for individuals LWO.

5.0 Common Bean Effects on Low-grade Inflammation in Obesity

5.1 The Obese Inflammatory Phenotype

Chronic systemic inflammation is a key component in the development of many diseases such as cardiovascular disease, metabolic syndrome, and diabetes mellitus [83] and is characterized by modest increases in pro-inflammatory cytokines [84]. White adipose tissue is the main site of initiation of obesity-associated inflammation, and as the quantity of adipose tissue increases, the secretion of pro-inflammatory cytokines increases as well [85]. Macrophages accumulate in adipose tissue in response to adipocyte hypertrophy and hypoxia due to adipocyte expansion, and these macrophages further intensify the inflammatory process via the production of proinflammatory cytokines [84]. Furthermore, tumor necrosis factor (TNF)-α activates the transcription factor nuclear factor (NF)-κβ, increasing the production of pro-inflammatory cytokines and resulting in increased expression of NF-κβ target genes (e.g., interleukin (IL)-6, TNF-α, and monocyte chemotactic protein (MCP)-1) [86]. Leptin and adiponectin are pro- and anti-inflammatory adipokines, respectively, that are produced by white adipose tissue. Leptin is increased in obesity and has been linked to increased size and number of adipocytes [87]. Adiponectin is reduced in obesity and is negatively correlated with body fat mass [88].

It has been proposed that microbial dysbiosis observed in obesity is associated with local and systemic inflammation, suggesting the presence of a relationship between microbiota composition and inflammatory status [29]. Research has shown a correlation between high-sensitivity-C-reactive protein (hs-CRP) and the F/B ratio, indicating the increased F/B ratio observed in obesity correlates to increased hs-CRP concentrations [29]. Furthermore, an impaired intestinal barrier integrity, also referred to as a “leaky gut”, contributes to inflammation and can occur in obesity [89]. The dysbiosis

observed in obesity has been associated with increased intestinal barrier permeability via alterations in the expression of tight junction proteins and mucin-producing cells [90]. Reduced intestinal barrier integrity means the intestinal cell wall has increased permeability, allowing for more pro-inflammatory mediators to cross into systemic circulation. An important intestinal-derived mediator of systemic and adipose tissue inflammation is lipopolysaccharide (LPS), whose plasma concentrations are elevated in obesity [91]. LPS is a component within the cell wall of Gram-negative bacteria that can "leak" through the intestinal barrier and into systemic circulation when the barrier's integrity is compromised, leading to inflammation [92]. One of the main ways LPS promotes systemic and adipose tissue inflammation is by signaling through toll-like receptor 4 (TLR4) on tissue macrophages, adipocytes, and other immune cells, which results in the production of pro-inflammatory cytokines, as described in detail by [92].

5.2 Common Beans Improve Aspects of the Obese Inflammatory Phenotype: Animal Trials

In rodent models, common beans have attenuated aspects of the obese inflammatory phenotype during the development of obesity (See **Table 2**). Animal models can provide additional insight on markers of adipose tissue inflammation that human trials can oftentimes not measure due to the invasiveness of retrieving tissue samples. In one study, 5-week-old male C57Bl/6 mice were fed a HFD (60% by kcal) or a HFD supplemented with 15.7% (wt/wt) navy bean powder for 12 weeks[46]. Inflammatory mediators were assessed in fasted serum and in white adipose tissue (epididymal (EAT)). Although body weight was not impacted by the navy bean diet compared to the HFD group, significant changes in circulating and EAT pro- and anti-inflammatory mediators were noted. For example, serum levels of leptin and adiponectin were also measured and showed that the navy bean powder elicited a decrease in the leptin/adiponectin ratio due to a significant increase

in serum adiponectin levels. Additionally, within EAT, bean diet reduced macrophage infiltration, mRNA expression of IL-6 and MCP-1, and activation of inflammatory transcription factors NF-κβ p65 and STAT3, while TNF-α and leptin remained unchanged between groups. Furthermore, EAT adiponectin mRNA was enhanced in mice consuming bean diet compared to HFD controls.

Serum and plasma concentrations of leptin in response to bean supplementation during diet-induced obesity have been reported by other studies. Serum leptin concentrations were reduced in male Wistar rats after consuming a HFD supplemented with 79.8% black bean powder for 2 months compared to a HFD control [47]. However, significantly lower body fat in rats fed the HFD supplemented with beans compared to the HFD control could be an explanation for the difference in leptin concentrations. As previously mentioned about this study, sucrose levels were higher in the HFD control group than the HFD bean group (7.8g vs. 0g/100g, respectively), therefore creating doubt in the reported decrease in serum leptin given changes in leptin can be a result of high glucose levels [93]. That being said, black beans have reduced plasma leptin concentrations following the consumption of 20% (wt/wt) black bean powder in addition to a purified HFD in male C57BL/6J mice [49]. Plasma leptin concentrations were 43.2% lower in the bean diet group than the HFD control. This feeding trial was 6 weeks long and mice were fed a HFD (46% by kcal), a HFD supplemented with 20% cooked black beans, or an LFD (16% by kcal). Body weight was not significantly different between HFD groups. After 6 weeks, plasma pro-inflammatory cytokines TNF-α, IL-4, -5, -10, -12, and interferon (IFN)-γ were significantly reduced in the bean diet group compared to the HFD control.

The above studies used HFD-induced obesity models where bean supplements were given during the development of obesity. Research has also shown the ability of common beans to improve the obese inflammatory phenotype after obesity has been established in mouse models (See **Table 2**). This is of importance as the established presence of inflammation is more reflective of the state of inflammation in individuals LWO. In mice with established obesity by feeding mice a HFD for 12 weeks, subsequent consumption of a HFD supplemented with 15.7% (wt/wt) navy bean powder led to significant reductions of EAT protein expression of IL-6, MCP-1, and macrophage inflammatory protein (MIP)-1α [51]. This was in comparison to a high-fat control diet, and the intervention lasted 8 weeks. EAT activation of NF-κβ p65 and STAT3 were also significantly reduced in bean-fed mice compared to the control diet. On the other hand, EAT protein expression of TNF-α and MIP-1β remained unchanged. As opposed to what has been observed during the development of obesity, bean supplementation did not result in significant changes to serum levels of leptin [49] or adiponectin [46]. Overall, beans are successful in modulating markers of inflammation, such as IL-6 and MCP-1, leading to anti-inflammatory effects in adipose tissue and systemically. The studies reviewed above vary in duration from 6-12 weeks and vary greatly in bean dose (15.7%-79.8%). Further studies should investigate if there are dose- or time-dependent effects of beans on markers of inflammation.

5.3 Common Beans Improve Aspects of the Obese Inflammatory Phenotype: Human Trials

Most studies investigating the ability of dietary pulses to modulate obesity-related inflammation prescribe a mixed variety of pulse types, making it challenging to determine individual effects of each pulse type. A summary of the studies presented and discussed in this review can be seen in **Table 2**. The following studies include participants LWO and dietary interventions comprised of

more than one pulse type. Individuals LWO that consumed 5 cups (approximately 896g) of yellow split peas, chickpeas, navy beans, and lentils per week for 8 weeks did not experience any changes in plasma levels of CRP or adiponectin [72]. Both pulse and control diets did significantly increase plasma leptin levels by 53% and 24% compared to baseline values, respectively. In another study, a combination of chickpeas, lentils, peas, and beans in addition to an energy-restricted diet successfully reduced pro-inflammatory markers in individuals LWO. Women and men LWO consumed a hypocaloric diet (-30% total energy expenditure) either with or without dietary pulses (4 servings/day) for 8 weeks [94]. Pro-inflammatory markers complement C3, hs-IL-6, hs-TNF-α, and CRP were measured in a fasted state pre- and post-intervention. Of these markers, hs-IL-6 was not changed by either dietary intervention. On the other hand, Complement C3 and hs-TNF-α were reduced by both diets, although the pulse diet produced a significantly greater reduction in complement C3. Both the pulse and energy-restricted diets resulted in significant reductions in body weight. After being adjusted for weight loss, the significant difference in complement C3 between diets remained. Specific to the pulse diet, CRP was significantly reduced from 2.7 ± 2.4 mg/l to 1.6 ± 0.9 mg/l. Conversely, another study reported unchanged fasted serum concentrations of CRP following an energy-restricted diet (-500 kcal) supplemented with 2 servings (1 cup) of pulses/day [95]. Participants in this study had abdominal obesity. Waist-to-hip ratio did not change over the duration of the intervention and body weight and BMI were not recorded. The pulses included in this 6-week intervention were cowpeas, chickpeas, split peas, lentils, and red, white, and wax beans.

Studies with dietary pulse interventions that expand BMI inclusion criteria to include those with overweight in addition to those with obesity have also reported alterations in inflammatory

markers. All three of the following studies to be discussed are randomized crossover study designs. At a dose of 250g beans/day, men with overweight and obesity at risk for colorectal cancer experienced a 20% reduction in serum hs-CRP levels from baseline levels [96]. Common beans prescribed in this 4-week intervention were navy, pinto, kidney, and black beans. Diets were separated by a 2-week washout period. A dose of 260g/day pinto beans and brown lentils/day in addition to a habitual diet for 6-weeks also found reductions in serum levels of hs-CRP [97]; however not to the same magnitude as Hartman *et al.* (2010) [96] despite both having diets with a similar dose of pulses. The pinto and brown lentil diet lowered hs-CRP concentrations by 4.86% ± 1.86% compared to baseline values in participants with overweight and a family history of diabetes [97]. Two potential explanations for the different magnitude of effect between studies may be due to the different populations (sex, health risks) and/or pulse types (4 bean varieties vs. 1 bean and 1 lentil variety). Both studies reported stable body weights and had 2-week washout periods. Another potential explanation is that the low-glycemic index diet prescribed by Hartman *et al.* (2010) [96] in addition to the pulses had an additional anti-inflammatory effect. Further research would be needed to determine whether the anti-inflammatory abilities of pulses are dependent on sex, health status, or mix of pulse varieties. A lower dose of 150g mixed pulses/day (lentil, chickpea, split pea, and bean varieties) for 8 weeks was unable to produce changes in serum levels of CRP in women and men with overweight and obesity [98].

Overall, research has been able to show that a variety of pulse types, including common beans, have the ability to elicit some anti-inflammatory effects on systemic markers of inflammation in individuals with overweight and obesity, although some results are conflicting [96–98]. Differences in sex, dose, and pulse type(s) should be taken into consideration when designing future studies.

Additionally, a greater variety of inflammatory markers need to be studied in order to fully understand the extent to which bean mediate inflammation in humans LWO.

5.4 Is the Effect of Beans on Systemic and Adipose Inflammation Mediated Through Their Effects on the Microbiota?

An explanation for how common beans may attenuate systemic and adipose tissue inflammation is through enhancing the integrity of the gut barrier. The intestinal wall is comprised of multiple layers and provides a physical and functional barrier that facilitates paracellular transport [99]. A healthy gut barrier has selective permeability to nutrients, metabolites, water, and bacterial products. An impaired intestinal barrier can allow for the passage of harmful contents into systemic circulation, resulting in systemic inflammation [99]. SCFAs have been shown to play an important role in maintaining the intestinal barrier, as reviewed by Liu *et al.* [100]. In particular, butyrate contributes to intestinal epithelial integrity by providing energy to intestinal epithelial cells and inducing colonic mucin expression [101,102]. Mucin is a main component of the mucosal layer of the intestinal barrier which serves as a protective physical barrier [103]. Furthermore, acetate, propionate, and butyrate promote gut barrier integrity by modifying the expression and distribution of tight junction proteins [104]. Tight junctions connect epithelial cells together to reduce paracellular movement across the intestinal epithelium, which is vital to the function of the barrier [105].

Certain bacterial species contribute to permeability and integrity of the gut barrier, meaning that the modulation of specific gut bacteria may lead to improvements in inflammation caused by increased intestinal permeability. It has been suggested that dysfunction of the epithelial barrier could be due to the loss of beneficial microbes. *Lactobacillus* and *A. muciniphila* are just a few

examples of gut bacteria with the ability to enhance the integrity of the gut barrier [43,106]. These bacteria have also been linked to obesity [26], and *A. muciniphila* abundance is modulated by bean consumption [45,46].

Inflammatory markers, such as IL-6, CRP, and TNF-α, are indicators of systemic inflammation[107]. As reviewed in Eslick *et al.* [107], SCFAs regulate inflammatory markers via activation of GPRs and through the inhibition of histone deacetylases (HDACs). SCFAs trigger downstream signaling cascades that inhibit inflammation by binding to GPR41 and GPR43 receptors [107]. SCFAs also reduce pro-inflammatory cytokine production by decreasing pro-inflammatory gene transcription via inhibition of HDACs [107]. The inhibition of HDACs reduces inflammation by attenuating the LPS-induced expression of NF-κβ-regulated cytokines [108]. NF-κβ is a transcription factor that regulates proinflammatory genes, and the NF-κβ pathway is a proinflammatory signaling pathway[108]. Polyphenols can reduce inflammation by affecting enzymatic and signaling systems involved in inflammatory processes, e.g., by inhibiting enzymes with pro-inflammatory properties[20]. Microbiota-derived phenolic metabolites have also been shown to have anti-inflammatory effects[20]. In summary, inflammatory markers are mediated by metabolites produced via fermentation of NDCs and polyphenols by gut bacteria.

6.0 Conclusion and Future Directions

This review focused on the ability of dietary pulses, specifically common beans (*Phaseolus vulgaris*) to alter the gut microbiome in obesity and the subsequent mediating effects this has on appetite regulation and chronic low-grade inflammation. A summary of the findings reported in this review can be seen in **Figure 1**. As shown in this figure, animal studies have shown a

consistent effect of beans on gut microbiota composition in mice both during and after diet-induced obesity. Human studies, on the other hand, are limited and provide an incomplete picture on the microbiota-modulating effects of beans. Although SCFAs are ligands for GPRs involved in the secretion of appetite hormones GLP-1, PYY, and ghrelin, there is still not enough, if any, evidence that this translates to measurable effects on energy intake in obesity (both rodents and humans). Lastly, common beans consistently demonstrate the ability to reduce inflammation in animal models of obesity by modulating inflammatory cytokines and reducing intestinal barrier permeability. This anti-inflammatory effect is also present in humans LWO; however, studies only report a limited number of inflammatory cytokines making it difficult to have a comprehensive understanding of this effect.

Further research is needed to determine consistent effects of common beans on the gut microbiome, appetite regulation, and inflammatory markers in individuals LWO. In order to do so, study designs need to include more comprehensive outcome panels, i.e., complete gut bacteria and microbial-derived metabolites, appetite hormones and sensations, as well as inflammatory mediators. In conclusion, there is some supportive evidence for the efficacy of common beans as a means to modulate the gut microbiome and improve appetite regulation and chronic low-grade inflammation in obesity, but more studies are required to determine a consistent effect specific to common bean consumption in humans LWO.

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Table 1. Gut microbiota structure and function after bean consumption in rodents and humans with obesity.

Reference	Subjects/Obesity Model	Duration	Pulse Diet(s)	Control Diet(s)	Gut Microbiota Method
Monk *et al.,* 2019 [46]	Male C57BL/6 mice; Obesity development	8 weeks	Purified HFD (60% kcal from fat) + 15.7% cooked navy bean powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (11% kcal from fat)	16S rRNA; Feces
Results:	Bean diet: ↑α-diversity, ↑*A. muciniphila,* ↑*Prevotella,* ↑*S24-7,* ↑*Sutterella,* ↑*Allobaculum,* ↑*Coprococcus,* ↓*R. gnavus,* ↓unassigned members of *Ruminococcaceae, Rikenellaceae, and F16* families; ↓unassigned members of *rc4-4, Lactococcus, Parabacteroides,* and *Adlercreutzia* genera; ↑Total SCFAs				
Neil *et al.,* 2019 [48]	Male NCI C57BL6/NCr mice; Obesity development	12 weeks	Purified HFD (60% kcal from fat) + 40% white kidney bean powder	Purified HFD (60% kcal from fat)	16S rRNA; Cecum
Results:	Bean diet: ↑*A. muciniphila,* ↓F:B ratio, ↓Firmicutes, ↑Bacteroidetes				
Lutsiv *et al.,* 2021 [45]	Male NCI C57BL/6NCr1 mice; Obesity development	17 weeks	Purified HFD + 40% lentil, chickpea, common bean, or dry pea powder	Purified HFD (60% kcal from fat)	16S rRNA; Cecum
Results :	All pulses : ↑α-diversity, ↑Bacteroidetes, ↓F :B ratio, ↓Proteobacteria, ~Actinobacteria, ↑*Muribaculaceae, B. acidifaciens, Rikenellaceae, Allobaculum, B. pullicaecorum, Sutterella,* Mogibacteriaceae (II)*, rc4 4,* and RF32; ↓*Oscillospira, R. gnavus, M. schaedleri, Dorea, C. methylpentosum, Lactococcus*, Peptococcaceae, Christensenellaceae, and *Streptococcus*; ~*Adlercreutzia, Bilophila*, Clostridiales (I), *C. hathewayi, Coprococcus*, Desulfovibrionaceae, Enterobacteriaceae, Erysipelotrichaceae, F16, *P. gordonii*, Ruminococcaceae (I), and *Ruminococcus* (of Lachnospiraceae). Bean diet : ↓Firmicutes, ↑*A. muciniphila*				
Sánchez-Tapia *et al.,* 2020 [47]	Male Wistar rats; Obesity development	2 months	1) Purified LFD + 79.8% dry cooked black bean (BB diet) 2) Purified HFD + 78% dry cooked black bean + 5% sucrose	1) Purified LFD (0% kcal from fat) 2) Purified HFD (35% kcal from fat)+ 5% sucrose	16S rRNA; Feces
Results :	Bean diets : ↑α-diversity, ↑Clostridia, ↑*Ruminococcus,* ↑*Coprococcus,* ↑*Prevotella,* ↓Total SCFAs, ↑Butyrate. BB diet : ↑Cyanobacteria, ↑Proteobacteria				
Tan *et al.,* 2021 [49]	Male C57BL/6J mice; Obesity development	6 weeks	Purified HFD (46% kcal from fat) + 20% black bean powder	1) Purified HFD (46% kcal from fat) 2) Purified LFD (16% kcal from fat)	16S rRNA; Feces
Results :	Bean diet : ~α-diversity, ↓F :B ratio, ↑Bacteroidetes, ~Firmicutes, ↑*Lachnospiraceae,* ↑*Muribaculaceae,* ↓*Blautia,* ↓*Clostridium sensu stricto 1,* ↓*Erysipelatoclostridium,* ↓*Romboutsia,* ↓*Turicibacter,* ↑*Ruminococcus 1*				
McGinley *et al.,* 2020 [50]	Male NCI C57BL/6NCr mice; Established obesity	12 weeks	Purified HFD + 40% common bean, chickpea, dry pea, or lentil powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (5% kcal from fat)	16S rRNA; Cecum
Results:	All pulses: ↑bacterial content, ↑Bacteroidetes, ↓F:B ratio. Bean and lentil diets: ↑*A. muciniphila,* ↓Firmicutes				

Weir *et al.,* 2020 [52]	Male NCI C57BL/6NCr mice; Established obesity	17 weeks	Purified HFD + 40% chickpea, common bean, dry pea, or lentil powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (5% kcal from fat)	16S rRNA; Cecum
Results:	All pulses: ↑α-diversity, ↑*A. muciniphila,* ↓F:B ratio				
Monk *et al.,* 2021 [51]	Male C57BL/6 mice; Established obesity	8 weeks	Purified HFD (60% kcal from fat) + 15.7% cooked navy bean powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (11% kcal from fat)	16S rRNA; Feces
Results:	Bean diet: ↓α-diversity, ↑A. muciniphila, ↑Bacteroidetes, ↑*Prevotella, ↑*Bacteroides, ↑S24-7, ↓Firmicutes, ↓Clostridiales, ↓*Ruminococcaceae, ↓Lactococcus ↓rc4-4*, ↑Total SCFAs				
Lutsiv *et al.,* 2022 [56]	Male and female C57BL6/J mice; Established obesity	12-14 weeks	Purified HFD (32.5% kcal from fat) + 0%, 10.2%, 20.4%, or 40.8% white kidney bean powder	Purified HFD (32.5% kcal from fat)	16S rRNA; Cecum
Results:	All doses (M&F): ↓F:B, ↓Firmicutes, ↓Actinobacteria, ↑Bacteroidetes, ↓ *B. pseudolongum, Dehalobacterium, Dorea, Lactococcus,* Ruminococcaceae (II), *Ruminococcus,* and *R. gnavus.* ↑*Clostridium (I)* and Rikenellaceae (I) All doses (F): ↓*Aldercreutzia,* Clostridiaceae (II), *Enterococcus, Clostridium (II), Lactobacillus (I), Streptococcus (I),* and Ruminococcaceae (I). All doses (M): ↓*AF12,* Clostridiaceae, *Lactobacillus, Allobaculum,* and *Parabacteroides.* 17.5% (M&F): ↓*Mogibacteriaceae, and* Lachnospiraceae (I) and (II). 70%(M&F): ↓Verrucomicrobia, ↑RF39				
	Human Studies				
Finley *et al.,* 2007 [59]	*n*=40M, 40F; BMI > 30 kg/m^2; Healthy and pre-metabolic syndrome; 2 x 2 factorial design	12 weeks	Habitual diet + 1 pre-made pinto bean entrée/day (130g cooked pinto beans/day)	Habitual diet + 1 pre-made isocaloric control entrée/day	Direct PCR; Feces
Results:	Bean diet: ↓*Eubacterium limosum*, ~*Bifidobacterium longum, Bacteroides vulgatus, Clostridium clostridiiforme, Methanobrevibacter smithii,* and *Peptostreptococcus productus*, ~Total SCFAs				
Sheflin *et al.,* 2017 [60]	*n*=12M, 17F; BMI 18-46.4kg/m^2; Colorectal cancer survivors; Randomized-controlled trial design	4 weeks	Habitual diet + 1 meal and snack made with 35g navy bean powder/day	1) Habitual diet + meal and snack made from 30g stabilized rice bran/day 2) Habitual diet + macronutrient matched meal and snack/day	16S rRNA; Feces
Results:	Bean diet (14 days): ~α-diversity, ~Firmicutes, ~Bacteroidetes, ↓*Bacteroides fragilis, ↑Lachnobacterium,* ~Total SCFAs Bean diet (28 days): ↑α-diversity, ~Firmicutes, ~Bacteroidetes, ↓*Bacteroides fragilis, ↓Clostridium, ↓Anaerostipes, ↑Lachnospira, ↑Coprococcus*, ~Total SCFAs				

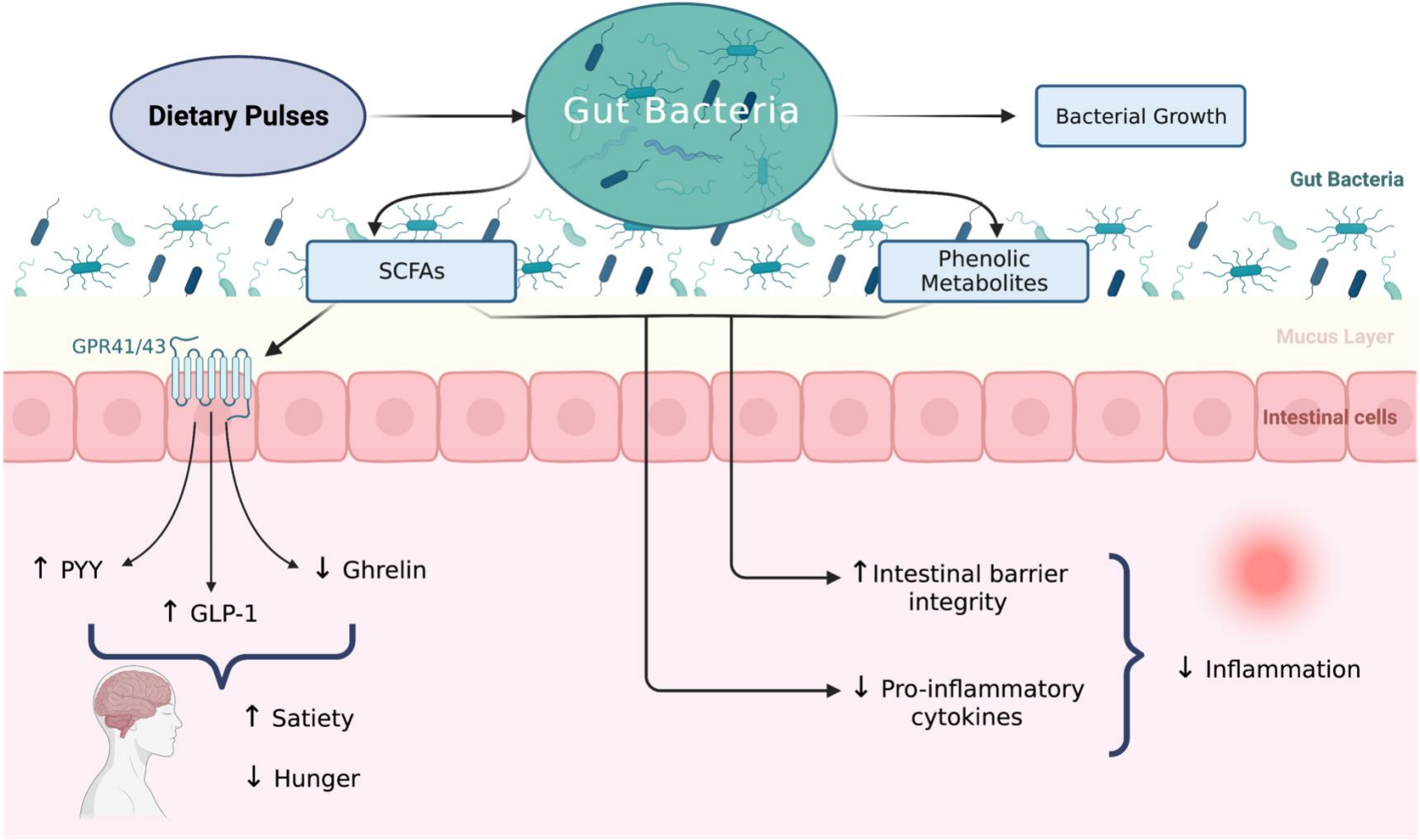
BMI (Body Mass Index; unit measures: Kg: kilograms / M^2: square meters); HFD (High fat diet); SCFAs (Short-chain fatty acids); *n* (number); M (Male); F (Female); F/B (Firmicutes/Bacteroidetes ratio); ~ represents no change.

Table 2. Inflammatory markers after bean consumption in rodents and humans with obesity.

Reference	Subjects/Obesity Model	Duration	Pulse Diet(s)	Control Diet(s)	Collection Method
Monk *et al.* (2019)[46]	Male C57BL/6 mice; Obesity development	8 weeks	Purified HFD (60% kcal from fat) + 15.7% cooked navy bean powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (11% kcal from fat)	Blood and EAT
Results :	Bean diet (blood): ↑adiponectin, ↓leptin:adiponectin ratio Bean diet (EAT): ↓NF-κβ p65, ↓STAT3, ↓MCP-1, ↓IL-6, ~TNF-α, ↑adiponectin, ~leptin				
Sánchez-Tapia *et al.,* 2020 [47]	Male Wistar rats; Obesity development	2 months	1) Purified LFD + 79.8% dry cooked black bean (BB diet) 2) Purified HFD + 78% dry cooked black bean + 5% sucrose	1) Purified LFD (0% kcal from fat) 2) Purified HFD (35% kcal from fat) + 5% sucrose	Blood and colon
Results :	Bean diets (colon): ↓NFκB. Bean diets (blood): ↓leptin				
Tan *et al.,* 2021 [49]	Male C57BL/6J mice; Obesity development	6 weeks	Purified HFD (46% kcal from fat) + 20% black bean powder	1) Purified HFD (46% kcal from fat) 2) Purified LFD (16% kcal from fat)	Blood
Results :	Bean diet: ↓leptin, ↓IL-4, -5, -10, -12, ↓TNF-α, ↓IFN-γ				
Monk *et al.,* 2021 [51]	Male C57BL/6 mice; Established obesity	8 weeks	Purified HFD (60% kcal from fat) + 15.7% cooked navy bean powder	1) Purified HFD (60% kcal from fat) 2) Purified LFD (11% kcal from fat)	Blood and EAT
Results :	Bean diet (EAT): ↓NF-κβ p65, ↓STAT3, ↓IL-6, ↓MCP-1, ↓MIP-1α, ~TNF-α, ~MIP-1β. Bean diet (blood): ~leptin, ~adiponectin				
			Human Studies		
Mollard *et al.* (2012)[72]	*n*=11M, 29F; Metabolic syndrome; Average BMI 32.8 ± 0.7 kg/m^2 Randomized parallel design	8 weeks	Habitual diet + 896g pulses/week; Pulses= yellow split peas, chickpeas, navy beans, and lentils	Energy-restricted diet (-500 kcal)	Blood
Results :	Pulse diet: ~adiponectin, ~CRP, ↑leptin				
Hermsdorff *et al.* (2011)[94]	*n*=17M, 13F; Average BMI 32.5 ± 4.5 kg/m^2 Randomized controlled trial	8 weeks	Energy-restricted diet (-30% total energy expenditure)+ 240-360g pulses/week; Pulses= lentils, chickpeas, peas, and beans	Energy-restricted diet (-30% total energy expenditure)	Blood
Results :	Pulse diet: ↓complement C3, ↓hs-TNF-α, ↓CRP, ~IL-6				

Safaeiyan *et al.* (2015)[95]	*n*=34F; Abdominal obesity; BMI not reported Randomized control trial	6 weeks	Energy-restricted diet (-500 kcal) + 1 cup pulses/day; Pulses= red, white, and wax beans, cowpeas, chickpeas, split peas, and lentils	Energy-restricted diet (-500 kcal)	Blood
Results :	Pulse diet: ~hs-CRP				
Hartman *et al.* (2010)[96]	*n*=64M; Average BMI 28.7 ± 3.5 kg/m^2 Randomized control trial	4 weeks	Low-glycemic index diet + 250g beans/day; Beans= navy, pinto, kidney, and black beans	High-glycemic index diet	Blood
Results :	Bean diet: ↓CRP				
Saraf-Bank *et al.* (2015)[97]	*n*=12M, 14F; Family history of diabetes; Average BMI 28.92 ± 0.85 kg/m^2 Randomized control trial	6 weeks	Habitual diet + 250g pulses/week; Pulses= pinto beans and brown lentils	Habitual diet	Blood
Results :	Pulse diet: ↓hs-CRP, ~IL-6, ~TNF-α, ~adiponectin				
Abeysekara *et al.* (2012)[98]	*n*=30M, 57F; Average BMI 27.5 ± 4.5 kg/m^2 Randomized control trial	8 weeks	Habitual diet + 150g pulses/day; Pulses= green and red split lentils, chickpeas, yellow split peas, and pinto, favo, broad, black, and kidney beans	Habitual diet	Blood
Results :	Pulse diet: ~CRP				

Note. BMI (Body Mass Index; unit measures: Kg: kilograms / M^2: square meters); HFD (High fat diet); LFD (Low fat diet); *n* (number); M (Male); F (Female); NF-κβ (nuclear factor-κβ); IL (interleukin); TNF (tumor necrosis factor); MCP (monocyte chemotactic protein); hs (high sensitivity); CRP (C-reactive protein); STAT3 (signal transducer and activator of transcription 3); IFN (interferon); MIP (macrophage inflammatory protein); EAT (epididymal adipose tissue); ~ indicates no change.


Dietary Pulses
Gut Bacteria
Bacterial Growth
Gut Bacteria
SCFAs
Phenolic Metabolites
GPR41/43
Mucus Layer
Intestinal cells
↑ PYY
↓ Ghrelin
↑ GLP-1
↑ Satiety
↓ Hunger
↑ Intestinal barrier integrity
↓ Pro-inflammatory cytokines
↓ Inflammation

Chapter 4: General Discussion

General Conclusion

This objective of this thesis was to summarize the current state of the literature regarding the effect of common bean consumption on the gut microbiome, appetite regulation, and inflammation in animal models and humans with obesity. More specifically, the potential efficacy of beans as a non-weight-centred treatment for obesity was explored. It was found that although there are a limited number of studies on this topic, a majority of studies found beans are capable of modulating the gut microbiome, appetite hormones/sensations, and inflammatory markers in both individuals LWO and in animal models of obesity. Animal studies are more abundant and consistent when it comes to microbial and inflammatory measures, possibly due to the ability to strictly control the environment. Human trials do show promise, however, as some studies show similar results to animal trials. The influence beans have on appetite regulation in obesity is perhaps the least studied of the three topics (i.e., gut microbiome, appetite regulation, and inflammation), both in animals and humans. The majority of short- and long-term appetite human trials mainly included participants with normal weight and lacked participants with obesity. Animal trials measuring appetite hormones[12,13] were also fairly limited in number. In theory, the high NDC and polyphenol content in common beans interact with gut bacteria in a way that alters the microbial community and produces microbial-derived metabolites that interact with appetite and inflammatory processes; however, more research is still required to see if these effects consistently translate to individuals LWO.

A narrative literature review was chosen for this thesis after unexpected delays due to COVID-19 restrictions made it unfeasible to continue with the original plan of conducting a randomized

controlled trial within the timeline of a master's thesis. Although the randomized controlled trial could not be fully executed, all preparation for the proposed trial was completed. I was involved in Ethics approval process and contributed to the preparation and packaging of both dietary supplements (bean and placebo powders) while waiting for the approval from the University to resume research with vulnerable populations. Additionally, I assembled all participant materials, including binders for data collection, while also running mock trials in preparation for actual data collection. I was responsible for posting recruitment ads on Campus, local businesses/recreation centres, and on social media. After a couple of weeks with no success in recruiting participants, it was decided to widen the sample population to both males and females, and to mitigate potential participant burden by reducing the intervention duration from 6 to 4 weeks. It was after the second round of recruitment that the decision was made to implement an alternative plan to fulfill the thesis requirements and so we decided to pursue with the narrative review included herein. Even though I have not conducted my own data collection, it is important to note that I have been intensely involved with another project currently ongoing in our laboratory over the last 5 months. In the context of this project, I have been responsible for multiple aspects of data collection including- body composition, food intake, cold exposure, indirect calorimetry, visual analog scales, and electromyography.

Strengths and Weaknesses

A main weakness of this thesis lies in the narrative nature of the literature review. There was no strict protocol used for the search, selection, or analysis process of the articles, resulting in a non-exhaustive and potentially biased list of articles. A systematic review would have produced a more accurate summary of the current body of literature, but this was also not possible with the timeline

for degree completion. However, relevant articles from systematic reviews published within the last two years[14,15] were extracted and discussed in this thesis. This allowed for the greatest number of articles applicable to this review to be included without employing a strict search strategy. This thesis was also able to combine available research measuring the gut microbiota and microbial-derived metabolites, appetite hormones and sensations, and inflammatory mediators into a single literature review. To the best of our knowledge this has not previously been done.

This review is also one of few to focus on a specific pulse type as opposed to dietary pulses as a group. It is important for researchers to analyze results based on pulse type because the degree to which pulses influence physiological measures may not be equal across pulse types due to variance in nutritional composition. Although this did disqualify many articles from this review, it allowed for a better understanding of the physiological effects of common beans. Animal trials consistently prescribed a single pulse type or analyzed results based on pulse type. Unfortunately, a large number of human trials prescribed mixed-pulse interventions without any distinction between pulse types, making it difficult to distinguish pulse-specific effects.

Future Implications for Research

Animal models of diet-induced obesity consistently showed that common beans can alter gut microbiota composition during both the development of HFD-induced obesity[12,16–19] and after establishing obesity[13,20,21], and while some human trials agree with this, more studies are needed to reach a consensus. The same can be said for the effect of beans on the obese inflammatory phenotype. More thorough measurements for both the microbiota and inflammatory markers are needed to show the full magnitude of effect in individuals LWO. For example, identifying the

whole microbiota community would give a more detailed depiction of gut microbiota composition versus targeting specific microbes. Furthermore, microbial-derived metabolites (i.e., SCFAs) should also be considered when analyzing the gut microbiome due to the large influence they have on appetite and inflammation.

Research on appetite regulation in response to a bean diet is sparse for both animal and human trials. In addition to the need for more studies in this area, it is important to include measurements that fully encompass the complexity of appetite control. Current animal trials show beans successfully modulate appetite hormones GLP-1 and ghrelin[12,13], but measurements of PYY have yet to occur. In human trials, it is not uncommon for only one measure of appetite (hormones or sensations) to be recorded. Future studies need to include measurements of appetite-related hormones (GLP-1, PYY, ghrelin), appetite sensations, and potentially energy intake to gain a more comprehensive understanding of the influence beans on appetite control. Additionally, current appetite-related human trials on both single meal and long-term consumption of beans lack participants LWO. The effect beans might exert on appetite regulation in individuals LWO cannot be determined until these individuals are consistently included in this line of research.

Sex- and dose-specific responses to bean supplementation has been shown in C57BL6/J mice with an established obese phenotype[22]. This is a fairly recent finding (2022) and most trials have yet to acknowledge any sex or dose related differences. Pulse Canada has recommended daily consumption of 100g pulses[23]; it may be beneficial for future studies to include multiple treatment groups prescribed varying doses to determine if benefits can been seen below this serving size, or if consumption over the serving size provides any additional benefits. Going forward, research

conducted with dietary pulse interventions should take potential sex- and dose-specific responses into account when developing the study design as well as during statistical analysis. Overall, a study that measures gut microbiota composition and function, appetite regulation, and inflammation in response to a common bean dietary intervention in individuals LWO, and that addresses the above concerns, is an appropriate next step to take.

Future Implications for the Public

The results from this literature review should be interpreted with caution as the research gathered was non-exhaustive and may not be fully representative of the current state of knowledge. That being said, the information gathered in this review can still be informative to individuals LWO, especially those looking for a health-centered intervention that is affordable and accessible. According to the 2021 Hunger Count Report by Food Banks Canada, visits to food banks have increased by 20.3% since 2019[24]. Socioeconomic factors and time constraints are just a few of the many barriers to obesity treatment[25]; for an intervention to be successful, it must reduce barriers such as these as much as possible. Beans address these identified barriers as they are a relatively inexpensive high-protein food source that can reduce grocery costs, help with food insecurity, and take little time to prepare. In addition to reducing barriers to treatment, beans create fewer greenhouse gas emissions compared to animal sources of protein[26]. Beans are a plant that do not require nitrogen fertilizers which is environmentally beneficial as nitrogen fertilization is a main driver of greenhouse gas emissions[27], and require fewer resources to produce compared to meat production[26]. In summary, recommending bean consumption to individuals LWO would be beneficial to not only their health, but also economically and environmentally.

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