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Gut microbiota and satiety regulation in human dietary intervention studies: A scoping review

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Astrid Leer-Salvesen

Abstract

Background: Obesity is rising worldwide, leading to increased risk of metabolic diseases and premature deaths. Understanding the complex factors that control hunger and regulate appetite is therefore important. The influence of gut microbiota on appetite control and the modulation of satiety through important gut hormones such as glucagon-like peptide-1, peptide YY, cholecystokinin, and ghrelin represents a novel and yet unexplored area of investigation. In addition, the impact of diet on gut microbiota and gut hormones is currently not fully explored.

Aim: The aim of this scoping review was to summarize recent research on the relationship between gut microbiota and satiety in human dietary randomized controlled trials.

Methods: This review employed a scoping method, with literature searches in Ovid EMBASE and MEDLINE as of September 2022 using keywords and MeSH related to "gut microbiota" AND "satiety regulation". Inclusion criteria were randomized controlled trials involving human participants, published between 2009 and 2022 written in English. Articles that did not assess gut microbiota and/or gut hormones, or those that did not involve food or supplements as interventions were excluded. Out of 142 articles initially found, 13 studies met the criteria. Metadata extracted covered publication details, study design, aims, methods, and main findings, regardless of primary and secondary outcomes. Quality assessment was based on the Critical Appraisal Skills Program.

Results: The 13 examined studies investigated the effects of dietary fiber, dietary supplements, and complex diets. Seven out of the 13 studies reported changes in both gut microbiota and gut hormones such as glucagon-like peptide-1, peptide YY, cholecystokinin, and ghrelin, whereas five studies did not report any changes in either gut microbiota or gut hormones, while one study observed changes only in gut microbiota. Alterations in Bifidobacterium were recurrent in four of the studies.

Conclusion: From the studies examined, various dietary factors including fiber, supplements, and more complex diets can potentially alter gut microbiota and satiety-regulating gut hormones. However, due to the heterogeneity of these studies, drawing clear conclusions about the impact of these dietary interventions on gut microbiota and satiety regulation is challenging. Further research is required to better understand the influence of fiber and diets on these aspects.

Sammendrag

Bakgrunn: Fedme øker over hele verden, og er forbundet med økt risiko for metabolske sykdommer og tidlig død. Det er derfor viktig med mer kunnskap rundt prosesser som regulerer sult og appetitt og metthet. Nye studier tyder på at tarmfloraen spiller en viktig rolle i denne reguleringen gjennom påvirkning av tarmhormoner som glukagonlignende peptid-1, peptid YY, kolecystokinin, og ghrelin. Selv om vi vet at kosten kan endre tarmfloraen, vet vi fortsatt lite om dette vil ha betydning for tarmhormoner og sult og metthetsregulering.
Mål: Målet med denne oppgaven var å oppsummere litteraturen som har undersøkt relasjonen mellom kosten, tarmmikrobiota og tarmhormoner i humane randomiserte kontrollerte studier.
Metoder: Det ble utført et litteratursøk i Ovid EMBASE og MEDLINE i september 2022 ved bruk av nøkkelord og MeSH-termer relatert til temaene "tarmmikrobiota" OG "metthetsregulering". Inklusjonskriterier var randomiserte kontrollerte forsøk utført på mennesker, skrevet på engelsk fra perioden 2009 til 2022. Artikler som ikke målte tarmmikrobiota eller tarmhormoner, eller de som ikke inkluderte kost eller kostfaktorer som intervensjoner, ble ekskludert. Av 142 artikler som opprinnelig ble funnet, møtte 13 studier kriteriene. Metadata dekket publikasjonsdetaljer, studiedesign, mål, metoder, og hovedfunn, uavhengig av primære og sekundære resultater. Kvalitetsvurderingen ble utført med Critical Appraisal Skills Program.
Resultater: De 13 studiene som ble evaluert undersøkte effekten av kostfiber, kosttilskudd og komplekse dietter. Syv av de 13 studiene rapporterte endringer både i tarmmikrobiota og tarmhormoner som glukagonlignende peptid-1, peptid YY, kolecystokinin og ghrelin, mens fem studier ikke rapporterte noen endringer verken i tarmhormoner eller tarmmikrobiota, og en studie rapportere kun endringer i tarmmikrobiota. Endringer i Bifidobacterium var tilbakevendende i fire av studiene.
Konklusjon: Fra de undersøkte studiene har ulike kost faktorer, som kostfiber, kosttilskudd og mer komplekse dietter, potensialet til å endre tarmmikrobiota og metthetsregulerende tarmhormoner. Det er imidlertid vanskelig å trekke en klar konklusjon om effekten av disse kostintervensjonene på tarmmikrobiota og regulering av metthetsfølelse på grunn av heterogeniteten i studiene. Det trengs flere studier for å bedre forstå effekten av fiber og dietter på tarmmikrobiota og regulering av metthet.

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Abbreviations

AgRP	Agouti Related Peptide
ARC	Arcuate Nucleus
AXOS	Arabinoxylan oligosakkarid
BMI	Body Mass Index
CCK	Cholecystokinin
GBA	Gut-Brain-Axis
GOS	Galacto-oligosaccaharides
GIT	Gastrointestinal tract
GLP-1	Glucagon-Like Peptide- 1
GLP- 2	Glucagon- Like Peptide -2
OXM	Oxyntomodulin
PYY	Peptide YY
RS	Resistant starch
SCFA	Short chain fatty acid
T2D	Type 2 diabetes
WHO	World Health Organization

1.0 Introduction

1.1 Obesity

According to the World Health Organization (WHO), more than 1.9 billion adults were overweight in 2016, of which more than 650 million were obese (World Health Organization, 2021). Obesity is a major risk factor for several diseases and thereby increases the risk of premature death (Safaei et al., 2021). Obesity is therefore considered a major public health concern and is ranked as the fifth foremost reason for death globally (Safaei et al., 2021). WHO defines obesity and overweight as abnormal or excessive accumulation of fat which can have a negative effect on health (World Health Organization, 2021). Body mass index (BMI) is a common method used to measure obesity, and BMI is calculated by dividing a person's weight in kilograms by the square of the height (kg/m^2). People with a BMI between 25 and 30 are defined as overweight, while BMI above 30 is considered obese (World Health Organization, 2021). The changing food environment and food culture have a major role in the recent rise in obesity (Pizarroso et al., 2021). The modern lifestyle, characterized by globalization and changes in dietary patterns, promotes a sedentary lifestyle and excessive food intake, resulting in energy storage and an increased risk of overweight and obesity (Pizarroso et al., 2021).

1.2 Satiety regulation

As research on obesity is quickly expanding, understanding the complicated factors that control hunger and the appetite regulation becomes important (Lean & Malkova, 2016). Satiety regulation refers to the process by which the body controls feelings of hunger and fullness in response to food intake (Blundell & Bellisle, 2013). Satiety involves a complex interplay between various hormones, neural signals, and behavioral factors (Blundell & Bellisle, 2013). The gastrointestinal tract (GIT) and the brain control essential functions of food ingestion and digestion through their interactions (Wren & Bloom, 2007). The GIT and the pancreas release hormones regulating satiety and body weight (Wren & Bloom, 2007). Hormonal and neural signals from the GIT are key players in this bidirectional signaling pathway (Tack et al., 2021). The biological system that contributes to appetite control has become better understood in recent years (Blundell & Bellisle, 2013). When food is not present in the GIT, hunger signals are sent, and food consumption is stimulated. Hunger is a

signal that starts the eating process, and this signal is produced in the stomach by the stimulation of the vagus nerve, which generates electrical signals, as well as the lack of food, enhanced by the secretion of the hormone ghrelin, and metabolic signals like hyperglycemia (Cabral et al., 2021). When food is present in the GIT, satiety signals override hunger signals, inhibiting further food intake (Tack et al., 2021).

1.3 Appetite-regulating hormones

Appetite and satiety sensations are regulated by the central nervous system and involve complex interactions between appetite and satiety regulators and the brain (Althubeati et al., 2022). Numerous peptides and hormones are believed to contribute to the short-term feelings of satiety and hunger (Lean & Malkova, 2016). More than 30 gut hormones, neuropeptides and neurotransmitters are now known to affect appetite (Lean & Malkova, 2016). These hormones may reduce food intake by decreasing hypothalamic orexigenic signaling, and increasing anorectic signaling (Sam et al., 2012). Ghrelin is an orexigenic peptide produced by the stomach which stimulates appetite and often referred to the as the “hunger hormone” (Druce et al., 2004). In contrast, glucagon-like peptide-1 (GLP-1), oxyntomodulin (OXM), Peptide YY (PYY), cholecystokinin (CCK), leptin, and pancreatic polypeptide (PP) are anorectic hormones that inhibits appetite (Druce et al., 2004). These peptides can also mediate inhibitory feedback mechanisms on intestinal transit, which contributes to prolonged gastric distension and increased satiety between meals (Sam et al., 2012). Satiety appears to be influenced by micronutrients, non-nutrients, and some bioactive food constituents. In addition to their direct digestive effects, macronutrients and micronutrients can have an indirect but powerful influence on satiety through their action on the gut microbiota (A. Tremblay & Bellisle, 2015). Among these hormones, those most extensively studied in relation to the potential influence of the gut microbiota include the satiety-regulating hormones GLP-1, PYY, CCK, and the hunger regulating hormone ghrelin (Chaudhri et al., 2006). Consequently, this review will focus on these hormones and their roles in appetite regulation.

Glucagon-like Peptide 1

GLP-1, a gut-derived hormone, is essential in regulating glucose metabolism and appetite, stimulating insulin secretion, inhibiting glucagon release, and slowing gastric emptying, thereby improving glycemic control and satiety (Sam et al., 2012). Produced by intestinal L-cells, its bioactive forms are released in response to an oral glucose load, promoting insulin

secretion and lowering blood glucose post-meal. Higher GLP-1 levels are linked to increased satiety and improved glucose tolerance (Canfora et al., 2017). Research indicates a possible interaction between GLP-1 and gut microbiota, with certain gut bacteria stimulating GLP-1 production through the fermentation of dietary fiber into short-chain-fatty acids (SCFAs) (Everard & Cani, 2014).

Peptide YY

Like GLP-1, PYY also slows down the rate at which food leaves the stomach and enters the small intestine. While both hormones play a role in inducing a feeling of satiety and reducing appetite, GLP-1 has a more prominent role in modulating insulin and glucagon release and regulating blood glucose (Zanchi et al., 2017). On the other hand, PYY has a significant function in slowing down gut motility and has been more directly associated with conditions like obesity due to its explicit role in appetite suppression (Karra et al., 2009). PYY concentrations are lowest during fasting but increases rapidly in response to food intake. PYY is also involved in the regulation of energy metabolism, insulin sensitivity, and glucose homoeostasis. PYY levels increase after a meal and promote a feeling of fullness by acting in the hypothalamus, the brain`s appetite control center, and the brainstem. This effect helps reduce food intake and can prevent overeating, thus contributing to weight management (Batterham et al., 2002). Low levels of PYY have been linked to obesity, while higher levels have been associated with lower body weight and lower risk of developing obesity-related diseases. The low levels of PYY could contribute to impaired satiety signaling, leading to overeating and weight gain (Batterham et al., 2002). Due to its role in appetite regulation, PYY has been investigated as a potential therapeutic target for obesity (Batterham et al., 2002).

Cholecystokinin

CCK is a hormone that is produced by cells in the lining of the small intestine and released into the bloodstream in response to the presence of food, particularly fatty foods (Moran & Kinzig, 2004). CCK plays a key role in the digestion and absorption of nutrients, particularly fat, and also affects appetite and satiety (Moran & Kinzig, 2004). CCK acts on the brain,

particularly the hypothalamus, to reduce hunger and increase feelings of fullness after a meal (Moran & Kinzig, 2004). CCK was the first gut-secreted peptide to be identified as a satiety factor, and contributes to decreased meal size (Ahima & Antwi, 2008). CCK is released postprandially from the small intestine and has been shown to co-localize with PYY in L cells (Sam et al., 2012). When CCK is released, it travels to the pancreas and stimulates the secretion of digestive enzymes, which help to break down fats, proteins, and carbohydrates in the small intestine (Sam et al., 2012).

Ghrelin

Ghrelin is called the "hunger hormone," and it has been shown to stimulate appetite and food intake (Sam et al., 2012). Although the stomach is the primary source of ghrelin production, small amounts of ghrelin are also produced in other parts of the body such as the brain, small intestine, and pancreas. However, the amount of ghrelin produced in these other parts of the body is much lower than that produced in the stomach (Sam et al., 2012). The level of ghrelin in the blood is high before meals and decreases after consuming food (Schalla & Stengel, 2020). Ghrelin has also been implicated in various pathological conditions, such as obesity, and obesity-related diseases (Cummings & Overduin, 2007).

There is growing evidence that the gut microbiota can affect the production and release of gut hormones, such as GLP-1, PYY, CCK, and ghrelin, which are involved in appetite regulation (Cani & Everard, 2016). The gut microbiota ferments dietary fibers, producing SCFAs such as acetate, propionate, and butyrate. These SCFAs can stimulate the secretion of gut hormones, like GLP-1 and PYY from enteroendocrine cells in the intestinal lining (Cani & Everard, 2016). The regulation of and physiological reaction to each meal is dependent on the total quantity eaten and glycemic control. A significant portion of these activities are believed to be mediated by ghrelin, CCK, GLP-1, and PYY secretion, as shown in Figure 1. (Steinert et al., 2017).

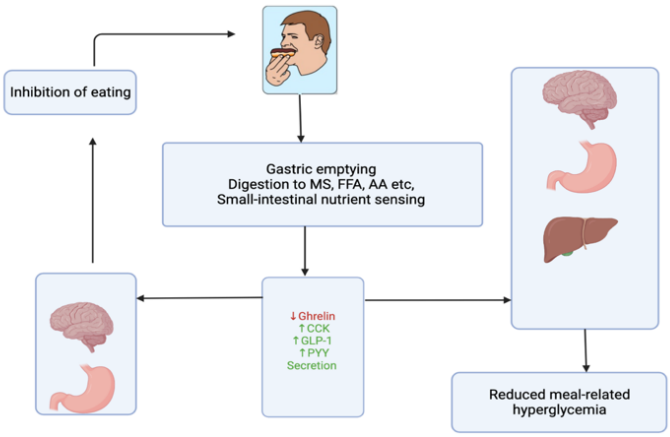

Figure 1: Overview of the physiological roles of ghrelin, CCK, GLP-1, and PYY in regulating eating and meal-related glycemia. The rate of gastric emptying, intestinal transit, digestion, and nutrient sensing in the small intestine primarily determine the inhibition of ghrelin secretion and the stimulation of CCK, GLP-1, and PYY secretion. These changes in hormone levels during and after meals lead to various gastrointestinal and central nervous system events that result in the inhibition of eating and the dampening of postprandial increases in outcomes, along with monosaccharides, free fatty acids, and amino acids, respectively (Made in biorender, adapted from Steinert et al., 2017).

Abbreviations: *CCK: cholecystokinin, GLP-1: glucagon-like peptide, PYY: peptide YY. MS: monosaccharides, FFA: free fatty acids, AA: amino acids*

Several studies have indicated that the composition of bacteria in the gut can play an important role in regulating body weight, appetite, and satiety regulation (Davis, 2016; Gomaa, 2020). Each species within the gut microbiota faces selective pressures based on the available nutrients and the presence of other bacterial species (van de Wouw et al., 2017). Every bacterial species strives to enhance its fitness, habitat, and survival by fermenting dietary nutrients in a specific manner and producing metabolites. Many of these metabolites can impact the host's appetite and eating habits by directly interacting with systems responsible for sensing nutrients and regulating feelings of hunger and fullness (van de Wouw et al., 2017). The gut microbiota is highly diverse and harboring trillions of microorganisms in human digestive system (Gomaa, 2020). Despite the established associations in this research, a direct causal relationship and the underlying mechanisms remain unclear (Maruvada et al., 2017). Research has shown that signals from the gastrointestinal tract can affect the central appetite-regulating area in the hypothalamus, often referred to as the "gut-brain axis (Han et al., 2021). Given that obesity has emerged as a significant public health concern, understanding the mechanisms that are involved in the human appetite regulation is important (Althubeati et al., 2022).

1.4 Gut Microbiota

There are numerous studies and reviews that have provided evidence on the role of gut microbiota in the development of obesity and associated health conditions (Cuevas-Sierra et al., 2019). Several studies have shown that the gut microbiota plays a crucial role in modulating the host`s energy metabolism, weight, and satiety, thereby contributing to the development of obesity-related diseases (Clemente et al., 2012; Delzenne et al., 2015; Diamant et al., 2011). Several studies have shown that the diversity in and richness of the gut microbiome are reduced in obese subjects (Denou et al., 2016; Heiss & Olofsson, 2018). Studies have consistently shown that obese individuals have a less diverse gut microbiota and a higher abundance of certain bacterial species, such as *Firmicutes*, and a lower abundance of others, such as *Bacteroidetes*, compared to individuals with normal weight (Gerritsen et al., 2011; Kumar et al., 2014). Studies have shown that a higher proportion of specific species, such as *Bacteroidetes* and *Akkermansia muciniphila*, is associated with increased production of SCFAs, which are involved in regulation of energy metabolism and satiety (Everard et al., 2013). However, there have been conflicting evidence related to the connection between host BMI and interindividual variations in the gut microbiota composition (Ridaura et al., 2013). It is important to note that the exact composition of the gut microbiota and its relationship to obesity may vary depending on various factors, including ethnicity, diet, and lifestyle (Maruvada et al., 2017).

The gut microbiota refers to the diverse community of microorganisms that inhabit the human gastrointestinal tract (GIT), primarily the large intestine (de Vos et al., 2022). These microorganisms, which include bacteria, viruses, fungi, and archaea, play an important role in digestion, immune function, and overall health (de Vos et al., 2022). All surfaces of the human body, including the GIT is colonized by microorganisms that together make up the body´s microbiota. The human gut microbiota is made up of 100 trillion microbes which exist in a largely symbiotic relationship with their human hosts, carrying at least 150 more genes (the microbiome) than the human genome (Gomes et al., 2018). The microbiome represents the sum of all genes present in the microorganisms that colonize a specific host organism (Barko et al., 2018). Most bacteria found in fecal samples from healthy human volunteers belong to two phyla, *Bacteroidetes* and *Firmicutes*, according to 16S rRNA-targeted molecular analyses (Ursell et al., 2014). The 16S rRNA-targeted molecular analysis is a

widely used technique for studying microbial communities and identifying bacteria (Ranjan et al., 2016). The gram-negative *Bacteroidetes phylum* comprises the genera *Bacteroides*, *Prevotella*, *Parabacteroides*, and *Alistipes*, whereas the gram-positive *Firmicutes phylum* includes *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Eubacterium hallii*, as well as many other low abundance species (Louis et al., 2010). Intestinal microbiota is critical for many biological processes in the human body including digestion, glucose metabolism and gut barrier function (Smits et al., 2021). The formation and expansion of the gut microbiome begins at birth, while the composition of the microbiota is mostly determined by genetic, dietary, medication, lifestyle, and environmental factors (Gomaa, 2020). The gut microbiota is known to regulate gut hormones and the production of SCFAs, both of which can influence satiety and appetite regulation (Allegretti et al., 2020). By promoting satiety, SCFAs may help prevent excessive calorie intake and thus contribute to maintaining a healthy body weight (Valdes et al., 2018). Individuals with a higher abundance of SCFAs-producing bacteria in their gut may be at lower risk of obesity-related disorders (Chambers et al., 2015). Intestinal bacteria ferment dietary fiber into SCFAs, including butyrate, propionate, and acetate (Woting & Blaut, 2016). These SCFAs can influence satiety by stimulating the release of GLP-1 and PYY, two hormones that help to reduce hunger and increase satiety (Woting & Blaut, 2016).

Given the importance of the gut microbiota in regulating energy metabolism and obesity, dietary approaches aiming at enhancing the abundance and activity of beneficial gut bacteria have become increasingly important (Davis, 2016). Diets high in fiber, prebiotics, and probiotics can help promote a diversity and healthy gut microbiota, which in turn may help reduce the risk of obesity related disorders (Leeming et al., 2019). Despite advancement in our understanding, several gaps in our knowledge remain. The direct causal relationships between gut microbiota and gut hormones are still unclear, and there is limited understanding of how variation in gut microbiota among individuals affect satiety regulation. There is also a need for more research on the effects of different interventions on gut microbiota and satiety regulation.

1.5 Effects of diets on the gut microbiota

Complex interactions between genetic background, gut microbiota, and diet have been reported as key factors influencing the risk of developing obesity and metabolic diseases (Cuevas-Sierra et al., 2019). The importance of diet in the gut microbiota modulation has

been widely recognized (Jeffery & O'Toole, 2013; Wu et al., 2016). These alterations in gut microbiota can lead to an increase or decrease in certain species, as well as alter production of metabolites in the gut environment (Gomaa, 2020). Macronutrients (carbohydrates, proteins, and fats), micronutrients (vitamins and minerals), fiber, prebiotics, and probiotics can all modulate the gut microbiota (David et al., 2014). Each dietary component can promote the growth of specific bacteria species, alter the metabolic activity of the microbiota, and ultimately affect the host. A diet high in fiber encourages the growth of fiber-degrading bacteria, leading to the production of SCFAs, which are beneficial (David et al., 2014). On the contrary, a diet high in fat and low in fiber can stimulate harmful bacteria's growth, resulting in toxic byproducts that may trigger chronic inflammation in the body (David et al., 2014). High intake of proteins and fats, particularly those from animal sources, has been linked with an increase in certain gut bacteria that can produce harmful metabolites. These metabolites are associated with various health problems, including cardiovascular disease and obesity (Tang et al., 2013). Furthermore, the gut microbiota influences the digestion and absorption of dietary nutrients and the production of hormones and neurotransmitters that regulate appetite, metabolism, and mood (Kho & Lal, 2018).

Modern diets, especially the Western-style diet high in saturated fat, sugar, and processed foods, and low in fiber and plant-based foods, have been associated with reduced bacterial diversity with a decrease in Bacteroidetes and an increase in Firmicutes, which may lead to health issues like obesity (Brown et al., 2012; Pizarroso et al., 2021). In contrast, long-term adherence to a Mediterranean diet rich in fruits, vegetables, whole grains, legumes, nuts, and healthy fats has been linked to a more diverse and healthy gut microbiota (De Filippis et al., 2016). Different long-term diets have been associated with distinct gut microbiota profiles, highlighting the importance of dietary habits in determining gut health. Even though the optimal composition of the gut microbiota is yet to be fully understood (Kashtanova et al., 2016), current research suggests that a diet rich in fiber and plant-based foods tends to promote a healthy gut microbiota (So et al., 2018). Garcia-Mantrana et al. demonstrated in a study that higher adherence to the Mediterranean diet is characterized by an increase in *Bifidobacteria* and a higher percentage of SCFAs (Garcia-Mantrana et al., 2018).
As a result, Mediterranean diet has a favorable effect on gut microbiota, particularly on its diversity and metabolic activity (Garcia-Mantrana et al., 2018). However, the long-term

impacts of different dietary patterns on gut microbiota are unclear, and further research is needed (Leeming et al., 2019).

Dietary fiber, an indigestible carbohydrate component found in plant material, is increasingly linked to beneficial health effects (Guan et al., 2021). Gut bacteria break down dietary fiber, resulting in the production of SCFAs like butyrate, acetate, and propionate, which are important for gut health (Makki et al., 2018). Prebiotics are a type of dietary fiber that serve as food for the beneficial bacteria in the gut. These substances are resistant to digestion in the upper part of the GIT, and reach the large intestine undigested, where they function as substrate (food sources) for microorganisms. The most well-known prebiotics are oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin. Prebiotics can also include other types of dietary fiber, like resistant starch (RS) (Makki et al., 2018). Prebiotics are naturally found in many foods, including oats, bananas, onions, chicory root, apples, artichokes, and certain types of whole grains (J. Slavin, 2013). Certain fibers might benefit some individuals, more than others, based on their unique gut microbiota (David et al., 2014). A diet high in fiber can foster the growth of beneficial bacteria like *Akkermansia*, *Bacteroides*, *Bifidobacterium* and *Lactobacilli* (Makki et al., 2018). While much is known about fibers` role in shaping the gut microbiota, the extent to which dietary interventions with fiber affect gut hormones is still unclear (Berding et al., 2021). The gut-brain-axis, a complex network linking the gut microbiota with the central nervous system, is pivotal in this context (Cryan et al., 2019). This network involves various metabolites, hormones, and neurotransmitters that modulate appetite control and energy balance, showcasing the intricate interplay between dietary fiber, gut microbiota, and overall health (De Vadder et al., 2014; Lin et al., 2012).

1.6 Microbiota-Gut-Brain Axis

The gut-brain axis is the physiological driver of satiation in humans (Lean & Malkova, 2016). Gut hormones, which are generated by enteroendocrine cells located throughout the gastrointestinal tract, are essential signaling molecules in the gut-brain axis (Sun et al., 2020). In gut-brain cross-talk, the relationship between gut microbiota and gut hormones has been extensively acknowledged (Montagnani et al., 2023; Sun et al., 2020). The microbiota-gut-brain axis (MGBA) is a bidirectional communication system between the gut microbiota, the enteric nervous system (which regulates gastrointestinal functions), and the central nervous

system (Cryan et al., 2019). A key aspect of the MGBA is the gut-brain signaling pathway, which involves the bidirectional communication between the gut and the brain through the vagus nerve, gut hormones and immune signaling molecules. For instance, the gut microbiota can modulate the production and release of neurotransmitters such as serotonin, which is involved in regulating mood, appetite, and pain perceptions (Cryan et al., 2019).

Further, the MGBA is pivotal in satiety regulation or the feeling of fullness post-eating (Cani & Everard, 2016). Through intricate interactions with gut hormones and the MGBA, the microbiota can influence satiety. This involves mechanisms like the production of SCFAs, which can stimulate hormones such as PYY, and GLP-1. These hormones, in turn, signal the brain to decrease appetite and enhance the feeling of fullness as shown in Figure 2. (Chambers et al., 2015).

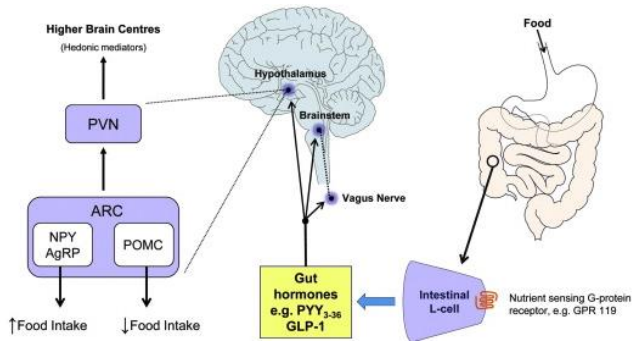

Figure 2: The gut-brain axis regulates food intake. G-protein coupled receptors on endocrine cells, such as L-cells, are activated by food-derived nutrients, leading to the release of gut hormones that can influence food intake at three locations: the vagus nerve, the brainstem, and the hypothalamus. Within the hypothalamus, the arcuate nucleus contains two neuronal populations critical for integrating peripheral signals and altering the drive to eat: the orexigenic neuropeptide Y (NPY)/ Agouti related peptide (AgRP) neurons and the anorexigenic proopiomelanocortin (POMC) neurons. There may also be additional connections between hypothalamic nuclei and higher brain centers that control the hedonic aspects of food ingestion. Key elements of this pathway include the Arcuate Nucleus (ARC), AgRP, GLP-1, paraventricular nucleus (PVN), and peptide YY (PYY) (Adapted from (Sam et al., 2012).
Abbreviations: *NPY- neuropeptide Y, AgRP- agouti related peptide, ARC- arcuate nucleus, PYY- peptide YY, GLP-1- gluckagon like peptide-1, POMC- proopiomelanocortin*

1.7 Exploring knowledge gaps

This introduction acknowledges the existing research gaps and sets the stage for the rationale behind choosing a scoping review for your study. Given the complexities and gaps regarding the relationship between gut microbiota and satiety regulation, the choice to conduct a scoping review is an appropriate method for identifying knowledge gaps where there is limited research in the area (Peterson et al., 2017). A scoping review is a method that present

an overview of the existing research on a selected topic (Arksey & O'Malley, 2005). Scoping reviews are used to examine broad themes that may contain many distinct study designs and methods, or to investigate an area that has not previously been thoroughly reviewed (Munn et al., 2018). Scoping reviews differ from systematic reviews in that they do not focus on narrowly-defined questions, but address broader topics (Arksey & O'Malley, 2005). Systematic reviews follow a predefined protocol to identify, select, and appraise all relevant literature on a specific research question (Munn et al., 2018). Research on gut microbiota and gut hormones includes a variety of study designs. The current scoping review methodology is inclusive of this diversity, allowing a more complete understanding of the topic. The interaction of gut bacteria and hormones is a broad topic, and unexplored area of investigation. A scoping review could be useful to draw connections between findings from different fields, which might be overlooked in more narrowly focused review types (Munn et al., 2018).

2.0 Aim of the study

The current scoping review aimed to summarize recent research on the relationship between gut microbiota and satiety regulation in human randomized controlled trials with the goal of identifying current state of knowledge in this field and highlighting areas for future research.

3.0 Methods

3.1 Methodological Approach

The method chosen for this study was a scoping review, which was particularly suitable considering the goal to summarize recent research on gut microbiota and satiety regulation. The current scoping review focused exclusively on RCTs, covering a diverse range of methodologies and study designs such as double-blind and crossover studies.

3.1 Search strategy

The search was carried out in OVID EMBASE and MEDLINE in September 2022 in close collaboration with an experienced librarian at Oslo Met. The keywords from the search in Ovid EMBASE were as follows “gut microbio*” or “intestine flora” or “microbiome” or “gastrointestinal microbio*” or “microbiota.mp.” AND “satiety” OR “satiety” or “hunger”, OR “satiety regulat.mp* OR “GLP-1.mp” OR “PYY.mp” OR “ghrelin” or ghrelin.mp OR “appetite”, OR “gut hormon.mp” OR “satiety hormone.mp” OR “cholecystokinin OR cholecystokinin.mp OR “CCK.mp”. The keywords from the search in Ovid MEDLINE were as follow “ intestine flora” OR “gut flora” OR “ gastrointestinal microbiome” OR “gut microbiota.mp” AND “satiety.mp” OR “satiety response” OR “satiation” OR “Glucagon like peptides” OR “glucagon like peptide.mp” “Peptide YY” or “PYY.mp” OR “Ghrelin” OR “ghrelin.mp” OR “Hunger” OR “gut hormon.mp” OR “Cholecystokinin” OR “CCK.mp” (Appendix 1 for detailed search string). Both keywords and MeSH terms were utilized in both databases to capture a broad range of relevant articles. OR and AND were used to combine search terms together. OR finds articles that contain one of the search terms, e.g., “gut microbiota or gut flora. AND finds articles that contain all of them, search terms e.g., “gut microbiota” AND “satiety regulation”. The inclusion criteria were as followed: randomized controlled trials (RCTs) conducted in humans, written in English, and published between 2009 and 2022. A total of 142 articles were identified through the initial search from Ovid EMBASE and MEDLINE. 142 articles were screened by title and abstract. After reading title and abstract 66 articles were excluded due to one or more of the following criteria: not original studies (reviews or meta-analysis), not RCTs, duplicates, conference reports, and studies on animals. After this exclusion, 76 articles were assessed for eligibility. 63 articles

were then excluded to due on or more of the following criteria: no data on gut hormones or/and gut microbiota, no dietary interventions with food and beverages. After applying the inclusion and exclusion criteria, 13 full-text articles were included in the current scoping review. The study selection process is detailed in a PRISMA flow chart (Figure 3).

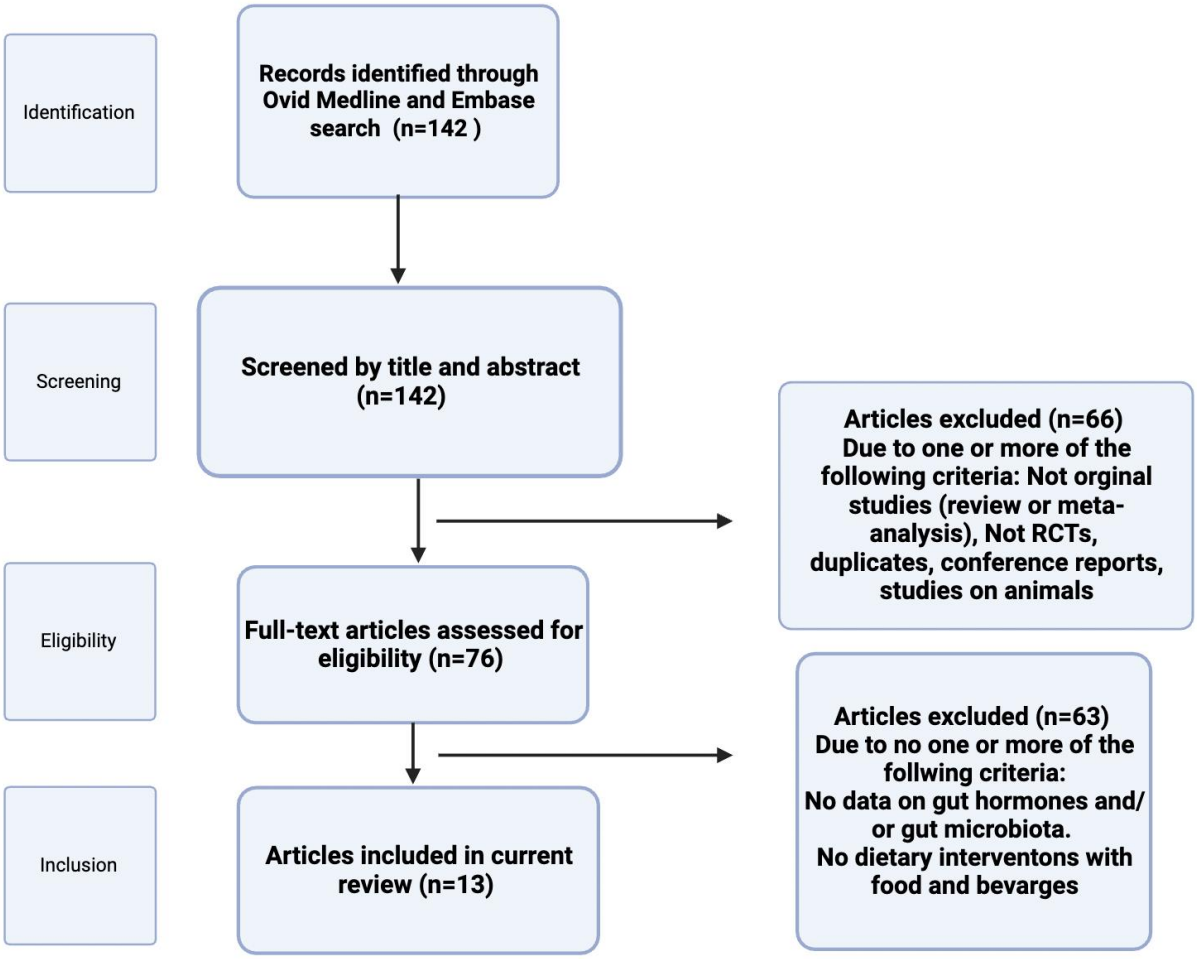

Figure 3:PRISMA Flow chart

3.2 Critical Appraisal Skills Programme (CASP)

Quality assurance of the reviewed studies was based on the Critical Appraisal Skills Programme (CASP) for RCT studies (*CASP Checklists - Critical Appraisal Skills Programme*, 2021). Critical appraisal is the process of systematically examining research evidence to judge its trustworthiness, value, and relevance in a particular context (Burls, 2015). The appraisal looks at the methodology, the statistical analysis, the results, and the

applicability of the studies (Burls, 2015). In this current scoping review each study was critically appraised using CASP RCT checklist, which consist of 11 questions (*CASP Checklists - Critical Appraisal Skills Programme*, 2021). The scores from CASP RCT checklist was primary used to support the credibility and reliability of the findings. All the reviewed studies have been evaluated using CASP and have received a score ranging from 1-11 (Table 1). The CASP RCT checklist is based on a critical review of each article using fixed formulated questions where one can answer either 'yes', 'no', or 'can't tell' (*CASP Checklists - Critical Appraisal Skills Programme*, 2021). Each 'yes' gave 1 point, while both 'no' and 'can't tell' gave 0 points in the assessment. As a result, the overall rating was calculated as a percentage of the total possible score. Within the assessment framework, this percentage provides a quantifiable measure of the study's methodological quality. The CASP checklist does not contain any designated point system recommended by the researchers behind the CASP tool. The scoring method applied in this scoping review was a modification introduced to keep track during the critical assessment of each individual study. For a detailed breakdown of the evaluations based on the CASP checklist for all included studies, refer to Appendix 2

3.3 Research ethics

Since this is a literature study included published articles, there is no requirement to apply to the Regional Ethics Committee (REK) or Sikt for approval. However, only studies of high ethical standards, and consistent with the Declaration of Helsinki, were included in this literature review.

4.0 Results

In the current scoping review, a total of 13 eligible RCTs involving human participants were included. These studies, carried out between 2009 and 2022, ranged in duration from 2 weeks to 9 months, and investigated the influence of gut microbiota and gut hormones in dietary intervention studies. The studies varied in methodology like the type of dietary interventions given, length of the intervention, sample size, study population and metabolic health. 16S rRNA gene amplification sequencing is used in most of the studies reviewed, while three of the included studies used qPCR to measure gut microbiota. 16S rRNA gene amplification sequencing is widely used method to study microbial communities, particularly in the context of bacterial populations (Caporaso et al., 2011). The majority of the studies used a temperature of -80 degrees Celsius for long term storage, but five of the studies deviated from this protocol, not freezing the samples within the recommended time frame. The quantification of gut hormones, including GLP-1, PYY, CCK, and ghrelin, was carried out using a range of advanced assay technologies. These technologies included Enzyme-Linked Immunosorbent Assay (ELISA), Radioimmunoassay (RIA), Luminex Multiplex Assays utilizing xMAP Bead-Based technology, and the Milliplex Human Metabolic Hormone Panel-Based Immunoassay. Each of these methods offers unique advantages in sensitivity, specificity, and multiplexing capabilities, allowing for precise and comprehensive measurement of these key gut hormones. ELISA is used in the majority of the studies. ELISA is a tool that is often used measuring gut hormones because of their sensitivity and specificity (Sakamoto et al., 2018). Over half of the reviewed studies had gut microbiota and gut hormones as secondary outcomes. Secondary outcomes are other, potentially relevant, parameters that are of secondary interest or are exploratory (Parker & Weir, 2022). The secondary outcomes may offer additional insights or help to explore additional hypotheses, but the study might not be statistically powered to explore these adequately (Wickham, 2019). This means that while secondary outcomes can provide useful insights and generate hypotheses for future research, they often need to be interpreted with caution. In the context of dietary interventions with fiber, supplements and complex diets, gut microbiota and gut hormone measurements were designated, and hence, the studies were not primarily designed to explore these aspects. The primary objectives for most of the reviewed studies were related to another outcome, such as changes in body weight, insulin sensitivity, blood glucose levels, glucose metabolism, or cholesterol levels.

The results from the included RCTs are systematically organized based on intervention type-fiber dietary interventions, dietary supplements, and complex diet, as summarized in Tables 2-4. This categorization was done to better understand the complex links between diverse dietary interventions and their impacts on gut microbiota and gut hormones. The overview shows the subject characteristics, study design, intervention, primary outcome, secondary outcome, gut microbiota, and gut hormones (Table 2-4). Each of the reviewed studies was scored against the CASP RCT checklist, as shown in Table 1. The CASP RCT checklist consists of 11 questions (*CASP Checklists - Critical Appraisal Skills Programme*, 2021). After assigning scores with CASP evaluation, none of the included RCTs scored below 72%. Scores ranged from 72% to 90% indicated that the research methods were robust and that the results were credible. (Table 1).

Table 1: Critical Appraisal Skills Programme (CASP) checklist score for the 13 included studies. (Number 1-13 indicates the studies)

CASP questions	1	2	3	4	5	6	7	8	9	10	11	12	13
Did the study address a clearly focused research question?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was the assignment of participants to interventions randomized?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were all participants who entered the study accounted for at its conclusion?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were the participants 'blind' to intervention they were given?	yes	yes	yes	yes	yes	no	yes	can't tell	yes	yes	no	yes	yes
Were the study groups similar at the start of the randomized controlled trial?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no
Apart from the experimental intervention, did each study group receive the same level of care	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were the effects of intervention reported comprehensively?	yes	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes
Was the precision of the estimate of the intervention or treatment effect reported?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Do the benefits of the experimental intervention outweigh the harms and costs?	yes	can't tell	can't tell	can't tell	can't tell	can't tell	no	yes	yes	yes	yes	yes	yes
Can the results be applied to your local population/in your context?	can't tell	can't tell	can't tell	can't tell	yes	can't tell	can't tell	yes	can't tell	yes	can't tell	can't tell	can't tell
Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?	yes	yes	yes	no	yes	yes	can't tell	can't tell	can't tell	can't tell	can't tell	yes	can't tell
Score	10/11 90% Very good	9/11 81% Good	9/1 81% Good	8/11 72% Good	9/11 81% Good	8/11 72% Good	8/11 72% Good	9/11 81% Good	9/11 81% Good	10/11 90% Good	8/11 72% Good	10/11 90% Very good	8/11 72% Good

Five of the studies investigated the impact of dietary fiber, like arabinoxylan-Oligosaccharide (AXOS), galacto-oligosaccharides (GOS), resistant starch (RS), Inulin and beta-glucan (Table 2), four explored the effect of various dietary supplements, including riboflavin, branched-chain amino acids (BCAAs), Moringa oleifera, and VSL#3 probiotics (Table 3), while four studies investigated the influence of complex diets, such as low-gluten diet, almond-based low-carbohydrate diet, weight maintenance diets versus weight loss diet, and white rice versus white bread diets (Table 4). The participant demographics ranged in age from 12 to 70 years and included both genders. Participants were adolescents or adults with diverse health statuses, from normal weight to overweight, obese, prediabetic, and type 2 diabetes. The participants' BMI ranged from below 18.6 kg/m² to 40 kg/m². The sample sizes of the studies ranged from as few as 7 to as many as 105 individuals, giving a total of 479 participants across all studies.

4.1 Effect on gut microbiota and satiety hormones after interventions with dietary fiber

Five of the included studies investigated the effect of fiber on gut microbiota and gut hormones (Table 2).

Muller et al. conducted a double-blind parallel trial that involved 48 normoglycemic healthy adults from the Netherlands with a BMI range from 20 to 30 kg/m² (Muller et al., 2020). The researchers were investigating whether the intake of AXOS could influence the whole-gut-transit-time (WGTT) in adults with slow GI transit but without constipation, this was the primary outcome for this study. Secondary outcomes were gut microbiota composition and gut hormones among others. Block randomization was used, and the researcher allocated 24 to receive AXOS, and 24 to receive placebo. Participants were given 15 g/day of AXOS as an intervention, while the control group received maltodextrin (placebo) for 12 weeks. Participants were instructed to consume the supplement or placebo once daily, preferably in the morning, and to maintain their regular diet and lifestyle throughout the study period. The gut microbiota was assessed using 16S rRNA gene amplicon sequencing with Hiseq2500. Feces tubes were stored at -20 degrees Celsius in the participants' freezers and transported using ice packs and immediately stored at -80 degrees Celsius on arrival. The administration of AXOS resulted in a significant reduction in alpha diversity and changes in the composition

of the gut microbiota compared to the placebo group. Specifically, the AXOS intervention was associated with a significant increase in beneficial gut bacteria, such as *Bifidobacterium*, *Akkermansia*, *Lactobacillus*, and *Prevotellaceae*. In contrast, there was a decrease in the abundance of *Blautia*, *Eubacterium Hallii* group, *Coriobacteriaceae*, and *Dorea*. Furthermore, they found a significant reduction in GLP-1 levels following the AXOS intervention compared to placebo group. No significant effect was observed on PYY levels. This was determined using different assays and kits like RIA kits, enzymatic assays, and ELISA kits (Muller et al., 2020).

In another double-blind, parallel trial, Canfora et al. investigated the impact of GOS on peripheral insulin sensitivity in 48 overweight and obese men and women from the Netherlands aged 45-70 years with a BMI from 28 to 40 kg/m² (Canfora et al., 2017). Secondary outcome included microbiota composition and gut hormones like GLP-1 and PYY. Participants were randomly assigned to receive either 15 g/day of GOS or a placebo (maltodextrin) for 12 weeks, in conjunction with their regular meals. Microbiota profiling was done using the Human Intestinal Tract Chip (HITChip), which is a phylogenetic microarray based on 16S ribosomal RNA gene sequences of over 1000 intestinal bacterial phylotypes. Fecal samples were collected at home a couple of days before the test days and were stored at -20 degrees Celsius. Upon arrival at the university, they were immediately stored at -80 degrees Celsius after being transported on dry ice. The study discovered that GOS significantly increased the abundance of *Bifidobacterium* in the gut microbiota compared to the placebo group. However, the overall microbial richness and diversity did not differ between the two groups. Beyond exploring the effects of GOS on gut microbiota composition, the study also examined how GOS influenced levels of gut hormones, specifically GLP-1 and PYY. By using RIA kits PYY was measured, and GLP-1 was measured with a specific assay. The authors found that there were no significant differences in GLP-1 and PYY levels between the intervention and placebo groups (Canfora et al., 2017).

Two studies were conducted by Canfora et al. from 2022 aiming to understand the effect of different fibers on SCFA production and their subsequent effects on metabolic parmaters in humans (Canfora et al., 2022). The study involved 45 participants from Maastricht Netherlands including lean participants (BMI 20 to 24.9 kg/m²), and those who were obese and prediabetic (BMI 25 -35.9 kg/m²). The age range was from 30 to 65 years old. Secondary

outcome for this study was microbiota composition. Two studies were conducted. In the inulin study, participants consumed either inulin with maltodextrin (INU), inulin with resistant starch (INU + RS) or a maltodextrin (placebo). In the beta glucan study, participants consumed either yeast beta glucan with maltodextrin (BG), BG with resistant starch (BG + RS) or maltodextrin (placebo). Randomization was peformed by an independent researcher using permuted block randomiztion. The inulin study included 23 participants, lean and obese, and the beta glucan study included 22 participants lean and obese. Following the intervention periods, the researchers examined the impact of the diets on gut microbiota and gut hormones. The researchers targeted the V4 region of the 16S rRNA gene for amplification and sequencing in the gut microbiota analyses. Fecal samples were collected in the morning on clinical investigation days and stored at -80 degrees Celsius. No significant changes were found in gut microbiota composition in inulin study or beta glucan study. Gut hormones were measured in blood collected in tubes treated with specific inhibitors. Total PYY measured using a specific monoclonal antibody and radioimmunoassay techniques. GLP-1 levels were measured using blood samples collected in tubes containing a dipeptidyl peptidase-IV inhibitor, and the samples were assayed for total GLP-1 immunoreactivity. The authors found no significant change observed for GLP-1 and PYY in the inulin study nor the beta glucan study (Canfora et al., 2022).

A randomized crossover study from 2019 included 19 participants with a BMI <24 kg/m² aged 18-55 years old from China to explore the effect of RS in body fat (Zhang et al., 2019). Secondary outcomes were gut microbiota composition and the gut hormones GLP-1 and PYY. The subjects were randomly divided into two groups by block randomization. In the study, 19 healthy Chinese individuals (10 women and 9 men) received 40g/day of RS as a supplement, followed by a crossover to an energy-matched control starch. The dietary intervention involved a high RS2 diet (72 g/day RS) and an energy-matched control starch diet (0g RS). Participants consumed it at a rate of 255.4 kcal/day (91.2g per day, containing 40g of RS. The placebo participants consumed it at a rate of 255.6 kcal/day (72g per day, containing 0g of RS). A uniform diet was designed and provided by the Department of Nutrition of Shanghai Jiao Tong University Affiliated Sixth People's Hospital to ensure that all subjects received almost the same food with equal overall macronutrients and caloric intake during the whole process, from the run-in period to the end of the trial. Both diets were given to the participants for two weeks each, with a two-week washout period in between.

Fresh fecal samples were collected using a commercial tube with DNA stabilizer and stored at -80 degrees Celsius prior to analysis. The researchers investigated the effect of RS on the gut microbiota composition at the genus level. V3 region of the 16S rRNA gene was targeted from each DNA sample. PCR amplification was then performed, and sequencing was carried out using the Roche Genome Sequencer. The authors found that 15 bacterial genera were significantly decreased: *Anaerostipes, Bacteroides, Blautia, Holdemanella, Coprococcus _1, Coprococcus _3, Erysipelotrichaceae, Eubacterium, Holdemanallea, Lachnoclostridium, Lachnospiraceae, Paraprevotella, Phascolarctobacterium,Ruminiclostridium, Ruminococcaceae_UCG-002* were significantly decreased, whereas *Ruminococcaceae_UCG-005* significantly increased. GLP-1 and total PYY were measured using quantitative ELISA kits. A significant increase was observed in GLP-1 at 30 minutes in a meal tolerance test after RS intake compared to the control group. RS consumption did not significantly affect the PYY level (Zhang et al., 2019).

The effect of various dietary fiber interventions on gut microbiota and gut hormones was assessed across five studies. The following observations refer to these specific five studies. Three out of the five studies demonstrated alterations in gut microbiota, while two of them reported a change in gut hormone levels. Two studies indicated no significant change in either gut microbiota composition or gut hormone levels. Notably, both AXOS and GOS were found to increase levels of the beneficial gut bacteria Bifidobacterium, which is recognized to be beneficial for gut health, according to the studies.

Table 2: Human studies investigating the effect of dietary fiber on gut microbiota and gut hormones

Study	Subject characteristics	Study design	Intervention	Primary outcome	Secondary outcome	Gut Microbiota	Gut hormones
Muller et al; 2020	N=44 22-55 y, normoglycemic adults with whole gut transit time of >35 h BMI: 20–30 Netherlands M/F	RCT Double-blinded , parallel trial 12 weeks	(1)15-gram arabinoxylan-oligosaccharides (AXOS) per day N=24 (2) Placebo (maltodextrin) N=24	Whole-gut-transit-time (WGTT) in adults with slow GI transit but without constipation	Gut microbiota composition and gut hormones GLP-1 and PYY	*Akkermansia* ↑ *Bifidobacterium* ↑ *Lactobacillus* ↑ *Prevotellacea* ↑ *Blautia* ↓ *Coriabacteriaceae* ↓ *Dorea* ↓ *Eubacterium Halli group* ↓	GLP-1 ↓ PYY ↔
Zhang et al; 2019	N=19 with normal body weight, 18-55 y Netherlands. BMI: <24 China M/F	RCT crossover 4 weeks	1)Probiotic fiber - Resistant starch (RS) (40-gram RS per day) (2) Energy-matched control starch	To explore the effect of Resistant Starch (RS) in boy fat.	Gut microbiota composition and the gut hormones GLP-1 and PYY	*Anaerostipes* ↓ *Bacteroides* ↓ *Blautia* ↓ *Coprococcus_1* ↓ *Coprococcus_3* ↓ *Erysipelotrichaceae* ↓ *Eubacterium* ↓ *Holdemanella* ↓ *Lachnoclostridium* ↓ *Lachnospiraceae* ↓ *Paraprevotella* ↓ *Phascolarctobacterium* ↓ *Ruminiclostridium_6* ↓ *Ruminococcaceae_UCG-002* ↓ *Ruminococcaceae UCG-005* ↑	GLP-1 ↑ PYY ↔
Canfora et al; 2017	N=44 overweight or obese prediabetic 45-70 y, (BM1: 28-40) Netherlands M/F	RCT Double-blinded, parallel 12 weeks	(1) 15-gram galacto-oligosaccharides (GOS) (2) Isocaloric placebo(maltodextrin)	Investigated the impact of GOS on peripheral insulin sensitivity	Microbiota composition and gut hormones GLP-1 and PYY	*Bifidobacterium* ↑	GLP-1 ↔ PYY ↔
Canfora et al; 2022	Study 1: N=23 N=12 lean BMI 20-24.9 N=11 obese prediabetic men BMI 25 - 35.9 30-65 y Netherlands M	RCT Crossover 4 weeks	Inulin study: (1)one day consumption inulin with resistant starch (INU+RS) (2) maltodextrin placebo	Gut hormone PYY	Microbiota composition	Changes in microbiota composition ↔	PYY↔
Canfora et al; 2022	Study 2: N=22 N= 11 lean BMI 20-24.9 N: 11 obese prediabetic men BMI 25 -35.9 30-65 y Netherlands M	RCT Crossover 4 weeks	Beta glucan study: (1)one day consumption of yeast beta glucan (BG) (2) maltodextrin placebo	Gut hormones	Microbiota composition	Changes in microbiota composition ↔	No changes

Significant differences ($p < 0.05$) between intervention group and control group are shown with ↑ or ↓ while ↔indicates no significant difference. The intervention group is referred to as (1), and the control group is referred to as (2). BMI: body mass index, F: female, M: male, GLP-1: Glucagon-like peptide 1, PYY: peptide YY

4.2 Effect on gut microbiota and satiety hormones after interventions with dietary supplements

Four of the included studies investigated the effect of different dietary supplements on gut microbiota and gut hormones (Table 3).

A double blind, parallel study conducted by Liu et al. studied the effect of two weeks with riboflavin supplementation (Liu et al., 2022). *Faecalibacterium prausnitizii* abundance, gut microbiota composition, and gut hormones were measured. Primary outcomes were the effect of riboflavin supplementation on *Faecalibacterium prausnitizii* abundance. Secondary outcomes included the microbiota composition and gut hormones. The participants consisted of 105 healthy adults from the Netherlands (males and females) with an BMI ranging from 18 to 25 kg/m². One group received 50 mg/d of riboflavin for two weeks, while another group received 100 mg/d of riboflavin for two weeks, and one group received placebo for two weeks. Duration of the study was 28 days (including 7-day run-in and a 7- day washout period). Participants collected fecal samples using stool kits. These samples were immediately frozen after collection and stored at −80 degrees Celsius until analysis. The storage duration was approximately 1 year ±4 months before they underwent sequence analysis. DNA was extracted from fecal samples, and the V3-V4 region of the 16S rRNA gene was amplified and sequenced. The study found that riboflavin supplementation did not significantly affect the alpha diversity or gut microbiota of the abundance of *Faecalibacterium prausnitizii.* Enzyme-Linked Immunosorbent Assays (ELISA) were used to measure GLP-1 and ghrelin. No significant differences were observed for GLP-1 or ghrelin (Liu et al., 2022).

Another dietary intervention, a double-blind parallel trial conducted by Gómez-Martínez et al. included 65 prediabetes participants from Spain, 40 to 70 years old with BMI below 35 kg/m² (Gómez-Martínez et al., 2021). The primary outcome for this study was to investigate if moringa oleifera supplementation can have a beneficial effect on blood glucose levels in individuals with prediabetes. Secondary outcomes were gut hormones like GLP-1, PYY and ghrelin and gut microbiota. The intervention group consumed daily six capsules of moringa oleifera, a plant with a high polyphenol content. The capsules contained 400 mg of moringa oleifera dry leaf powder. While the control group received a placebo capsule (microcrystalline cellulose). The intervention lasted for 12 weeks. Participants were instructed to take two

capsules before each main meal (breakfast, lunch, and dinner), every day through the intervention. The study population had prediabetes as per the American Diabetic Association (ADA) criteria. This meant they had HbA1c levels between 5.7–6.4%, fasting glucose between 100–125 mg/dL, or glucose levels between 140–199 mg/dL after a 2-hour glucose tolerance test. The participants provided a fecal sample collected one or two days before each visit. Subjects provided a fecal sample collected one or two days before each visit. These samples were immediately frozen and stored at −80 degrees Celsius until analysis. SYBR-Green real-time PCR was performed for the detection of 16S rRNA genes using specific primers targeted to various bacterial groups, such as *Bacteroides, Bifidobacterium spp., Lactobacillus spp.,* and others. Standard curves for each qPCR assay were used to quantify target bacterial DNA in the fecal samples. Gut hormones involved in appetite control, such as GLP-1, ghrelin, and PYY, were analyzed using xMAP Luminex technology with the Human Metabolic Hormone magnetic bead panel technology. Specific protease inhibitors were added to prevent the degradation of active ghrelin and GLP-1 before centrifugation. The authors found no significant changes in the abundance of gut microbiota groups between moringa oleifera intervention and placebo groups by using 16S rRNA gene sequencing. In addition, the study found no significant changes in the levels of gut hormones, including ghrelin, PYY, and GLP-1, between the moringa oleifera intervention and placebo groups (Gómez-Martínez et al., 2021).

Genton et al. examined in a crossover study the effects of branched-chain amino acids (BCAAs) supplementation and glycine on gut microbiota and gut hormones in 27 hemodialysis patients from Switzerland. This group consisted of male and females with a BMI 27.7 ± 5.1 kg/m², and age 61.2 ± 13.7 (Genton et al., 2021). The intervention was one BCAAs pack contained a mix of amino acids - 3.62 g leucine, 1.45 g isoleucine, and 1.94 g valine. Participants were randomized to take either glycine or BCAAs for 4 months, then underwent a 1-month washout period, and then took the other supplement for another 4 months. Patients recevied either a BCAA supplement twice daily (7g each time) for 4 months, followed by a 1-month washout period before crossing over to glycine supplement for antoher 4 months. Participants consumed two packs daily, one before breakfast and another before lunch. Supplements were given 30 minutes before breakfast and the 30 minutes before lunch. Participants were advised to continue their usual diet and physical activity throughout the study period and were monitored for any adverse effects or changes in their health status.

Patients provided stool samples, which were immediately stored in their fridge at 2–8 degrees Celsius and transported to the laboratory within 24 hours. For the metataxonomic analysis of fecal microbiota, DNA was extracted from stools using the ZymoBIOMICS DNA Miniprep Kit. The V3–V4 region of the bacterial 16S rRNA genes was amplified and sequenced. The authors found that overall microbiota diversity did not change signficantly with glycine or BCAA supplementation. However, the supplementation did lead to a significant decrease in the abundance of *Bifidobacterium dentium* and *Lacticaseibacillus paracasei* with the BCAAs supplementation. Serum samples were used to assess appetite mediators, which included total ghrelin, active ghrelin, GLP-1, CCK and PYY. The analyses of gut hormones were performed by enzyme-linked immunosorbent assay (ELISA) using different assay kits. GLP-1, PYY and CCK increased after BCAAs intervention compared to placebo (Genton et al., 2021).

Jones et al. conducted a double-blinded parallel study to investigate the effects of VSL#3 Probiotic supplementation on gut microbiota and gut-derived appetite-regulating hormones. This study involved 19 obese Latino teenagers aged 12 to 18 years old, males and females with BMI percentile ≥95th for age and gender (Jones et al., 2018). Primary outcomes were changes in gut microbiota, and gut appetite regulating hormones which include GLP-1, PYY, and ghrelin. The secondary outcomes for this study were changes in body composition, liver fat, liver fibrosis, plasma levels of insulin and glucose, and food intake. The study lasted for 16 weeks, and participants were randomly assigned to receive either three packets of VSL#3 probiotics daily or matched inactive product (placebo) that was designed to be similar in taste but contained no active probiotic cultures. Participants were randomly assigned to either the treatment (probiotic) or control (placebo) group. Randomization was carried out using an "adaptive stratified block design" to ensure a balanced distribution of sex between the two groups. Stool samples were collected using kits from Second Genome. The kits contained a preservative, and samples were stored at −80 degrees Celsius upon receipt. The relative abundance of bacteria taxa in the stool samples was determined through 16S rRNA Amplification Sequencing. The results showed that the gut microbial composition was not significantly altered by the probiotic supplementation. After intervention, the gut microbial composition did not show any significant alteration due to the probiotic treatment. The following gut bacteria that were measured: *Bacteroidetes, Cyanobacteria, Euryarchaeota, Firmicutes, Fusobacteria, Lentisphaerae, Proteobacteria, Tenericutes, and Verrucomicrobia*. Blood samples were collected, processed, and plasma was obtained. The plasma levels of gut

hormones, including active and total ghrelin, active GLP-1, and PYY, were determined using these ELISA kits. The study did not find any significant changes in gut hormones GLP-1, PYY or ghrelin between the groups after the intervention (Jones et al., 2018).

The effect of various dietary supplements intervention on gut microbiota and gut hormones was assessed across four studies. The following observations refer to these specific four studies. In summary, out of the four studies with dietary supplements, only the one involving BCAAs supplementation demonstrated alterations in both gut microbiota and gut hormones. The other three studies did not report significant effects on either gut microbiota composition or levels of gut hormones.

Table 3: Human studies investigating the effect of dietary supplements on gut microbiota and gut hormones

Study	Subject characteristics	Study design	Intervention	Primary outcome	Secondary outcome	Gut Microbiota	Gut hormones
Liu et al; 2022	N=105 27-33 y, BMI 18-25 Netherlands M/F	RCT Double blind, parallel trial 2 weeks	(1) 50mg/d riboflavin (2)100 mg/d riboflavin group (3) Placebo	Effect of riboflavin supplementation on Faecalibacterium prausnitzii abundance	Microbiota composition and gut hormones	*Faecalibacterium prausnitzii* ↔ Gut bacterial diversity ↔	GLP-1 ↔ (Compared with placebo and Ribo50 groups).
Gomez-Martinez et al; 2021	N=65 Prediabetes 40-70 y BMI ≥35 Spain M/F	RCT Double blinded, parallel trial 12 weeks	(1) Moringa oleifera capsules n=31 (2) Placebo n=34	To explore if Moringa oleifera supplementation can have a beneficial effect on blood glucose levels in individuals with prediabetes	Gut hormones like GLP-1, PYY and ghrelin and gut microbiota	*Blautia coccoides* ↔ *Eubacterium rectale* ↔ *Bacteroides fragilis* ↔ *Clostridium cluster IV* ↔ *Bifidobacterium* ↔ *Enterobacteriaceae*↔ *Lactobacillus group* ↔ *Faecalibacterium prausnitzii* ↔ *Akkermansia muciniphila* ↔ *Enterococcus spp* ↔	Ghrelin ↔ PYY ↔ GLP-1 ↔
Genton et al; 2021	N=27 Haemodialysis patients, Mean Age 61.2 ± 13.7 Mean BMI 27.7 ± 5.1 Switzerland M/F	RCT crossover study 9 months	Branched amino acids (BCAAs) N=15 Glycine N=12 28 days wash-out period crossover	Impact in gut microbiota	Gut hormones GLP-1, PYY and CCK	*Bifidobacterium-dentium* ↓ *Lacticaseibacillus paracasei* ↓	GLP-1 ↑ PYY ↑ CCK ↑
Jones et al; 2018	N=19 obese 12-18 y BMI percentile ≥95th for age and gender Hispanic adolescent M/F	RCT Double blinded, parallel trial 16 weeks	(1)Three packets per day of VSL#3®Probiotic n=8 (2)Placebo n=11	Changes in gut microbiota, and gut appetite regulating hormones which include GLP-1, PYY, and ghrelin	Changes in body composition, liver fat, liver fibrosis, plasma levels of insulin and glucose, and food intake.	*Actinobacteria* ↔ *Bacteroidetes* ↔ *Cyanobacteria* ↔ *Euryarchaeota* ↔ *Firmicutes* ↔ *Fusobacteria* ↔ *Lentisphaerae* ↔ *Proteobacteria* ↔ *Tenericutes* ↔ *Verrucomicrobia*↔	GLP-1 ↔ PYY ↔ Ghrelin ↔

Significant differences ($p < 0.05$) between intervention group and control group are shown with ↑ or ↓ while ↔ indicates no significant difference. The intervention group is referred to as (1), and the control group is referred to as (2). BMI: body mass index, F: female, M: male, GLP-1: Glucagon-like peptide 1, PYY: peptide YY, CCK: Cholecystokinin

4.3 Effect on gut microbiota and satiety hormones after interventions with complex diets

Four of the included studies investigated the effect of complex diets (Table 4).

In a crossover study by Johnstone et al., they aimed to examine the effects of nondigestible carbohydrates, particularly resistant starch type 3, on weight maintenance (WM) after a weight loss (WL) period. Secondary outcomes include changes in fecal microbiota composition, microbial metabolite concentrations, and gut hormones (Johnstone et al., 2020). 19 volunteers from London aged 20 to 62 years with BMI ranging from 27 to 42 kg/m² were included in the study. They had no known medical conditions or medications that could influence their appetite or mood. Initially, they followed a 3-day maintenance diet with 15% protein, 30% fat, and 55% carbohydrates. This was followed by a 21-day weight loss (WL) diet with 30% protein, 30% fat, and 40% carbohydrates. Subsequently, participants were put on two 10-day weight maintenance (WM) diets in a crossover design without washout periods. These WM diets had 20% protein, 30% fat, and 50% carbohydrates, but differed in resistant starch type 3 (RS) content: the RS-WM diet provided 22g/day for females and 26g/day for males, while the control WM diet (C-WM) had no RS. Subjects were provided with a breakfast test meal on four occasions, at the end of each dietary phase. This corresponds to the mornings of days 8, 29, 39, and 49. Fecal samples were taken on various study days, stored at 4 degrees, and processed within 5 hours. After processing, the relevant text mentions that the extracted DNA and other aliquots from the samples were stored at −70 degrees Celsius until further analysis. 16S ribosomal RNA amplicon sequencing was performed to analyze the composition of gut microbiota. The RS-WM diet led to a significant increase in *Ruminococcus bromii*, and a significant increase in the percentage of *Faecalibacterium prausnitzii* was also observed after the RS-WM diet, compared to WL-diet. Regarding gut hormones the researchers found that ghrelin increased significantly after the WL diet period relative to the WM containing RS diet and WM diet. For measuring gut hormones in the study, the researchers used a bead-based immunoassay, specifically the Milliplex Human Metabolic Hormone Panel from Millipore Corp. There were no significant changes in gut hormones GLP-1 and PYY, but the WL diet group had significantly higher ghrelin levels compared to the other diets (Johnstone et al., 2020).

Ren et al. conducted a parallel study with 45 participants from China over 18 years old diagnosed with type 2 diabetes (Ren et al., 2020). The BMI for the almond based low carbohydrate group (LCD) was 23.53 ± 2.33, and 23.69 ± 2.83 for low-fat diet group (LFD). The study aimed to assess the effect of an LCD-diet on depression in patients with Type 2 Diabetes Mellitus (T2DM). Secondary outcomes were gut microbiota composition and fasting GLP-1 concentration. Control Group (placebo) adopted an LFD-diet. The LFD diet was based on a six-point formula developed by the researcher team according to diabetes dietary guidelines. The intervention LCD group consumed 56 g/day of almonds replacing 150 g/d staple food (rich in carbs) from their diet. The rest of the dietary regimen was the same for the control group. Participants in the intervention group were provided with specific instructions on almond consumption. Participants were instructed to consume almonds between meals, with breakfast, or when they were hungry. Participants underwent a one-week washout period during which they did not consume nuts for at least 4 days. Fecal specimens from all participants were collected. Roughly 20 g of fresh feces were taken from participants using a sterile cotton swab and stored in sterile feces collection tubes. These samples were immediately placed in a portable liquid nitrogen tank for flash freezing and then transferred to a −80 degrees Celsius refrigerator for longer-term storage. For each fecal specimen, DNA was extracted and purified. The V3-V4 region of the 16S rRNA genes was amplified using PCR with modified universal bacterial primers. The researchers assessed the gut microbiota and GLP-1 levels at baseline and after three months, comparing the results between the two groups. Using 16S rRNA gene sequencing, they found that the LCD diet significantly increased the abundance of *Roseburia, Ruminococcus,* and *Eubacterium* compared to LFD group. Additionally, by the third month, the level of *Firmicutes* was significantly lower in the LCD group compared to the LFD group. *Bacteroides* also decreased significantly in the LCD group compared to baseline levels. GLP-1 was measured using enzyme-linked immunosorbent assay (ELISA). The fasting peripheral venous blood of participants was collected in vacuum blood vessels containing EDTA anticoagulants. To prevent GLP-1 degradation, DPP-4 inhibitors were added immediately after blood collection. The blood samples underwent centrifugation, and the supernatant was stored at appropriate cold temperatures. A significant increase in GLP-1 was observed in the LCD group (Ren et al., 2020).

Hansen et al. conducted a crossover study investigating the effects of a low-gluten diet on the intestinal microbiome of 60 healthy adults from Denmark aged 22 to 65 years old, with a BMI between 25 to 35 kg/m², without any known disorders (Hansen et al., 2018). The primary outcome was the alteration in gut microbiota composition. Secondary outcomes were blood markers such as gut hormones like GLP-1 and PYY. Participants were given diets that either limited daily gluten intake (~2 g/day) or increased it (~20 g/day). The intake was compared with a national average of gluten consumption in Denmark. During interventions, participants replaced their usual cereal products with study-provided low-gluten or high-gluten products. The intervention lasted for 8 weeks, with a washout period of at least six weeks in between. Fecal samples were collected in the morning of each of the four examination days, and immediately stored at 5 degrees Celsius for a maximum of 24 h before equal volume of sterile water was added and the sample was homogenized. The homogenized sample was aliquoted to cryotubes and stored at −80 degrees Celsius. To quantify certain bacteria, such as *Bifidobacterium spp.*, quantitative PCR (qPCR) was performed. The habitual intake for this population was 12 g/day. They found that compared to a high-gluten diet, a low-gluten diet induced moderate changes in the intestinal microbiome. A total of 575 species were identified, out of which the abundance of 14 bacterial species was altered during the low-gluten diet intervention when compared with the high-gluten diet intervention. Using metagenomics sequencing they found that specifically, the abundance of four species of *Bifidobacterium* was diminished during the low-gluten diet. The low-gluten diet led to a reduction in the abundance of four species of *Bifidobacterium*. *Blautia wexlerae*, *Dora longicatena*, *Eubacterium hallii*, two species of *Anaeostipes hadrus*, and *Eubacterium* significantly decreased. Simultaneously, certain unclassified species from the *Clostridiales* and *Lachnospiraceae* families increased during the low-gluten diet. Notably, the authors did not find any changes in alpha or beta diversity. In regard to gut hormones, plasma was analyzed using the Milliplex Human metabolic hormone panel bead-based immunoassay from Millipore Corp. The low-gluten diet was associated with a higher postprandial PYY response compared to the high-gluten diet, although no changes were observed in GLP-1 levels (Hansen et al., 2018).

Mano et al. examined the effect of two major Japanese staple foods, white rice and white bread, on gut microbiota and satiety in a study of seven healthy participants (Mano et al., 2018). Seven healthy volunteers, student and research staff at Kyoto University, Japan were

recruited, 31 to 42 years old with a BMI ranging from 18.6 to 23.1 kg/m²,. The primary outcome for this study was changes in the abundance of fecal *Bifidobacterium.* Secondary outcomes included GLP-1 levels. The study recruited healthy volunteers, mainly students from the research department, technical and research staff. This was a randomized crossover trial. The study started with a 1-week run-in period. Subjects were randomly assigned in a 1:1 ratio to one of two intervention sequences. First Sequence: Participants consumed a bread-based diet with supplied side dishes for one week, followed by a rice-based diet with supplied side dishes for the next week. Second Sequence: Participants consumed a rice-based diet with supplied side dishes for one week, followed by a bread-based diet with supplied side dishes for the next week. Washout Period: A 1-week washout period was included between the two test periods to minimize carryover effects from the first dietary period to the second. During the run-in and washout periods, subjects were advised not to consume probiotics, yogurt, oligosaccharides, and cultured milk drinks. In the test periods, the subjects' diet was restricted to staple foods (either white bread or white rice) and a specific set of supplied side dishes. The nutritional content of these foods was carefully calculated and controlled. Subjects collected fecal samples at home, immediately stored them with dry ice, and then brought them to the laboratory. Fecal samples were collected by participants at home. After collection, they were stored in boxes with dry ice (−78 degrees Celsius) and subsequently brought to the laboratory. The samples were stored at -80 degrees Celsius until analysis. 16S rRNA gene sequencing was employed to analyze the microbial community structure in these samples using a MiSeq device. The sequencing targeted the V3–V4 region of bacterial 16S rDNA. While no significant differences were found in the abundance of *Bacteroides* and *Firmicutes* between the two diets, the abundance of *Actinobacteria* was found significant higher after the bread period compared to the rice period. At genus level *Bifidobacterium* was more abundant after the bread period compared to the rice period, and at species level *Bifidobacterium longum* was significantly higher after the bread consumption period compared to the rice period. Conversely, the abundance of *Blautia faecis* was significantly higher after the white rice period. In terms of gut hormones GLP-1 was measured by using ELISA kits. GLP-1 was significantly higher after the bread consumption period compared to white rice period (Mano et al., 2018). The effect of various complex diets intervention on gut microbiota and gut hormones was assessed across four studies. The following observations refer to these specific four studies. In summary, all four of the studies demonstrated alterations in gut microbiota and gut hormones.

Table 4:Human studies investigating the effect of complex diets on gut microbiota and gut hormones

Study	Subject characteristics	Study design	Intervention	Primary outcome	Secondary outcome	Gut Microbiota	Gut hormones
Johnstone et al; 2020	N=19, 20-62 y, BMI 27–42 London M/F	RCT crossover 49 days	3-day maintenance diet (WM) with 15% protein, 30% fat, and 55% carbs, then a 21-day weight loss (WL) diet with 30% protein, 30% fat, and 40% carbs. Next, they were on two 10-day WM diets with 20% protein, 30% fat, and 50% carbs, differing in resistant starch type 3 (RS) content	Weight maintenance	Changes in fecal microbiota composition, and gut hormones GLP-1, PYY and ghrelin	*Faecalibacterium prausnizii* ↑ *Ruminococcus bromii* ↑ (after a RS-WM-diet compared to after a WL diet)	GLP-1 ↔ PYY ↔ Ghrelin ↑ (During WL diet)
Ren et al; 2020	N=45, >18 y, T2DM BMI: LCD group: 23.53 ± 2.33 LFD group: 23.69 ± 2.83 China M/F	RCT parallel trial 3-months	(1) An almond based low carbohydrate diet n=22 (56 g/day of almonds replacing 150 g/d staple food) (2) Low fat diet n= 23	Effect of an almond-based Low Carbohydrate Diet on depression in patients with Type 2 Diabetes Mellitus (T2DM).	Gut microbiota composition and fasting GLP-1 concentration	*Eubacterium* ↑ *Roseburia* ↑ *Ruminococcus* ↑ *Bacteroides* ↓ *Firmicutes* ↓	GLP-1 ↑
Hansen et al; 2018	N=60, 22-65 y, BMI 25-35 kg/m2 without known disorders M/F Denmark	RCT crossover 8 weeks	(1) Low gluten diet (2-gram gluten per day mainly from oats) (2) High gluten diet (18-gram gluten per day, mainly from wheat and rye) Wash out period 6 week (12gram gluten per day)	Gut microbiota composition	Gut hormones PYY and GLP-1	*Anaerostipes hadrus* ↓ *Bifidobacterium (4 species)* ↓ *Blautia* ↓ *Dorea longicatena* ↓ *Eubacterium* ↓ *Lachnospiraecea (2 species)* ↓ *Anaeostipes hadrus (2 species)* ↓ *Eubacterium hallii* ↓ *Clostridial* ↑ *Lachnospiraecea*↑	PYY - ↑ GLP-1 ↔
Mano et al; 2018	N=7 healthy subjects, 31-42 y, BMI 18.6-23.1 Kyoto, Japan M/F	RCT crossover 2 weeks	1-week intervention of either white rice or white bread, each with 21 frozen side dishes, followed by a 1-week washout period, and then switched to the other staple for another week	Changes in the abundance of fecal Bifidobacterium genus	Gut hormones GLP-1	*Bacteroides* ↔ *Firmicutes* ↔ *Blautia faecis* ↑ (Higher in rice period compared to bread period) *Actinobacterium Bifidobacterium*↑ *Bifidobacterium longum* ↑ (Higher in the bread period compared to rice period)	GLP-1 ↑ (Higher after consuming bread than after rice)

Significant differences ($p < 0.05$) between intervention group and control group are shown with ↑ or ↓ while ↔indicates no significant difference. The intervention group is referred to as (1), and the control group is referred to as (2). BMI: body mass index, F: female, M: male, GLP-1: Glucagon-like peptide 1, PYY: peptide YY.

5.0 Discussion

5.1 Methodological discussion

The current review has been made as transparent as possible, presenting all steps made throughout the processes. The use of a PRISMA flow chart, as presented in Figure 3, illustrate the study selection procedure underscores the commitment to transparency and replicability. The literature search was carried out in two well-known databases, Ovid EMBASE and MEDLINE, in September 2022. This approach was designed to expand the search scope and capture a diverse range of relevant RCTs. The defined inclusion criteria align with the study's objectives, targeting RCTs involving humans' subjects conducted from 2009 until 2022, published in English. The inclusion criteria employed was helpful to guide, save time, minimize mistakes, and guarantee transparency and reproducibility (Muka et al., 2020). The exclusion criteria was also clearly defined, dismissing non original studies like reviews, meta-analyses, conference papers, animal studies, and duplicates, and RCTs that did not have foods, drinks, or dietary supplements as intervention. Articles were excluded if they did not measure either gut microbiota, gut hormones, or both, as these measurements were essential for the aim of the current scoping review. When choosing the search terms, the objective was to identify studies that align with the aim of examining gut microbiota and satiety regulation in RCTs. The initial search yielded 142 articles from both Ovid EMBASE and MEDLINE which were then screened based on the predefined inclusion and exclusion criteria. Key words and Mesh terms were used. An advantage by using MeSH terms is that all articles that have been tagged with a specific topic can be captured, even if the keyword that has been used is not mentioned in the article (Richter & Austin, 2012). However, we cannot rule out the possibility that there are studies investigating the impact of diet on gut microbiota and satiety that are not included in the current scoping review due to the lack included Mesh terms. The comprehensive searches were conducted in collaboration with an experienced librarian at Oslo Met. This assured a more thorough search by using the librarian's experience in the search process. Despite these advantages, it is essential to recognize that no search approach is without limitations. It is always possible that some significant studies were missed. Also, some certain search terms may have been ignored, which can be a weakness of this scoping review. To ensure even broader coverage, future research or reviews may explore expanding the search to other databases, using more search methods, and other search terms.

A scoping review was conducted to explore the effect of diet and dietary components on gut microbiota and satiety regulation in human RCTs. Scoping reviews are meant to provide a broad overview of a topic, with the purpose should be to map the literature, identify gaps, and offer a comprehensive summary rather than to answer a very specific research question (Munn et al., 2018). An advantage of this methodology is its capacity to integrate a wide variety of study designs and provide an expansive overview of a given research area. This is particularly useful when the topic is complex or has not been extensively reviewed (Munn et al., 2018). In this current scoping review, it enabled the examination of the existing evidence without focusing solely on data synthesis, which is common in systematic reviews (Arksey & O'Malley, 2005). Given the limited studies investigating the relationship between gut microbiota and satiety regulation, the choice to conduct a scoping review was an appropriate method for identifying knowledge gaps where there is limited research in the area (Arksey & O'Malley, 2005). A scoping review is a rigorous process with a purpose that is different from systematic reviews. The motivation behind conducting the present scoping review stems from the knowledge gap in existing literature, as a limited number of studies have investigated the effect of diets on gut microbiota and satiety regulation in RCTs. The inclusion criteria to only include RCTs was based on the high-quality evidence that RCTs normally provide. Randomized controlled trials (RCTs) have traditionally been regarded as the gold standard of clinical trial design (Zabor et al., 2020). Randomization method can reduce differences in group characteristics that may influence the outcome, providing the most conclusive evidence regarding the impact of the exposure or intervention on the outcome (Zabor et al., 2020). By including only RCTs, the present scoping review emphasizes the importance of quality evidence. Although the inclusive nature of scoping reviews permits the engagement with a wide range of study designs, a critical reflection on this review must acknowledge that its insights are derived from a relatively limited pool of 13 RCT articles.

The methodology deployed also incurred a potential bias through its reliance on a single reviewer in the identification and examination of the included articles. The human error factor becomes more prominent without an additional set of eyes to double-check findings, data extraction, and evaluations. Using several reviewers can enhance the robustness and reliability of a literature review. However, since the review of the included articles was conducted alone, articles were discussed throughout the entire process with supervisors to

ensure a comprehensive understanding and thorough evaluation. During the entire process, two supervisors have regularly reviewed the work, provided valuable feedback, and presented opposing opinions. The choice of search words and MeSH terms, inclusion and exclusion criteria were discussed with supervisors to ensure that the aim could be met. Articles were also discussed when there were doubts if they could be included or not.

More than over half of the included studies had gut microbiota and gut hormones as secondary outcomes and there may therefore be some limitations when interpreting the findings related to the effect of diet on gut microbiota and gut hormones. Secondary outcomes are other, potentially relevant, parameters that are of secondary interest or are exploratory (Parker & Weir, 2022). The secondary outcomes may offer additional insights or help to explore additional hypotheses, but the study might not be statistically powered to explore these adequately (Wickham, 2019). This means that while secondary outcomes can provide useful insights and generate hypotheses for future research, they often need to be interpreted with caution. In this scoping review, which focuses on dietary interventions involving fiber, supplements, and complex diets, the primary objective of most studies was to examine changes in body weight, insulin sensitivity, blood glucose levels, glucose metabolism, and cholesterol levels. In the studies where measurements of gut microbiota and gut hormones were designed as secondary outcomes there might be some limitations when interpreting the findings related to these variables.

Traditionally, scoping reviews do not include a quality appraisal of the evidence, but rather scoping reviews contain existing literature without weighing the evidence (Arksey & O'Malley, 2005). However, to ensure the rigor and relevance of the RCTs included in this scoping review, a CASP evaluation was performed for all the included studies. This was done to critically appraise the methodological quality and reduce potential biases associated with the interpretation of these RCTs within the broader context of the review. It could enhance the depth of the review by providing a supplementary layer of insight into the quality of the existing literature (Munn et al., 2018). The CASP assessment is a reputable tool used to evaluate and appraise the quality of research articles, ensuring that they meet a certain level of validity, reliability, and relevance (Long et al., 2020). Including criteria for quality assessment like CASP checklist adds transparency to the current scoping review. The checklist were used to consider aspects such as randomization, blinding and the results (Long et al., 2020). In

terms of the CASP evaluation for RCTs as summarized in Table 1 all the included studies had a clearly focused research question, and all the studies had randomized their participants to the interventions, which strengthens the validity of the results. Most studies implemented blinding, but this was not always possible in the crossover trials with dietary intervention. The studies appeared to have a clearly defined protocol, outlining the participant characteristics, intervention details, and the outcomes that will be measured. Almost all of the studies undertook a power calculations that are essential in determining the sample size needed for the study to detect an effect of a given size (Serdar et al., 2021). P-values were reported in all the included studies, as well as confidence intervals. The variability in outcomes observed across the studies cannot be attributed to the quality of the research, as indicated by the CASP scores. After conducting CASP evaluation of the selected studies, a summarize of the scores reflected the RCTs methodological rigor, relevance, and overall quality. The CASP scores, ranging from 72% to 90%, are reflective of personal evaluation criteria, and indicate a generally high quality of the included RCTs. A "good" rating, defined by the CASP score of >70%, implies studies that have met the majority of the quality criteria in relation to the CASP evaluation. With this in mind Critical appraisal is about more than just ticking off items on a checklist. While the CASP can provide an indication of quality, it alone cannot capture the full complexity and nuances of research quality (Munn et al., 2018). Although checklists are intended to standardize evaluation, different users may interpret the questions differently, which can lead to variability in how articles are appraised.

There are many ways to analyze gut microbiota (Allaband et al., 2019). While most studies employed similar methodologies, primarily 16S rRNA gene sequencing to study gut microbiota, differences in data processing, sequencing depth, and primer choices can create variations in the results (Z. Liu et al., 2008; J. Tremblay et al., 2015). The 16S rRNA gene sequencing technique is commonly used because it is cost-effective and technically less complex (Jovel et al., 2016). However, it has its limitations, such as the inability to distinguish between closely related microbial species and strains, and it does not provide information about the functional capabilities of microbial communities (Jovel et al., 2016). Previous research has highlighted that significant variability can be introduced during both DNA extraction and PCR amplification stages in studies utilizing 16S rRNA applications (Brooks et al., 2015). The process of extracting DNA and amplification by PCR can cause some inconsistency in the results, especially when using 16S rRNA applications (Klindworth

et al., 2013). Different parts of the 16S rRNA gene provide different levels of information, and the primer used may prefer certain bacterial taxa over others. This means the final representation of the bacterial community might not be completely accurate due to these factors (Klindworth et al., 2013). There is a recommended protocol of freezing samples immediately or within a few hours of collection to ensure microbiome stability (Sinha et al., 2016). However, five of the reviewed studies did not freeze the samples within the recommended time frame, which can lead to skewed results. In almost all of the reviewed studies, gut hormones were measured by using ELISA (Enzyme-Linked Immunosorbent Assay), which is a common method used to detect and measure the concentrations of proteins, such as hormones, in samples (Albrechtsen & Rehfeld, 2021). The inclusion of other methods like Radioimmunoassay (RIA), Luminex Multiplex Assays, and the Milliplex Human Metabolic Hormone Panel-Based Immunoassay demonstrate the diversity of available techniques. Each of these methods has its unique advantages and limitations, which can affect the study outcomes.

5.2 Result discussion

The current scoping review summarizes the impact of dietary fiber, dietary supplements and complex diet on gut microbiota and satiety regulation. To ensure structure and clarity, the results were organized into tables (2-4). The 13 examined studies investigated the effects of dietary fiber, dietary supplements, and complex diets. Seven out of the 13 studies reported changes in both gut microbiota and gut hormones such as glucagon-like peptide-1, peptide YY, cholecystokinin, and ghrelin, whereas five studies did not report any changes in either gut hormones or gut microbiota, and one study reported changes only in gut microbiota. Alterations in *Bifidobacterium* were recurrent in four of the studies. PYY levels remained unchanged in most of the studies, except of two studies that demonstrated a significant increase in PYY. Variations in GLP-1 levels were noted across different studies: while one study reported a decrease, others observed an increase, and several studies found no change in GLP-1 levels.

Impact of diet and dietary components on gut microbiota

Eight of the 13 studies reviewed demonstrated an alteration in gut microbiota after intervention with fiber, supplements and complex diets (Canfora et al., 2017; Genton et al., 2021; Hansen et al., 2018; Johnstone et al., 2020; Mano et al., 2018; Muller et al., 2020; Ren et al., 2020; Zhang et al., 2019). The current scoping review demonstrates that interventions with fiber like AXOS, GOS, and RS and complex diets have the possibility to increase several bacterial species, including *Akkermansia, Bifidobacterium, Blautia, Eubacterium, Faecalibacterium prausnizii, Firmicutes, Lactobacillus, Lachnospiraceae, Prevotellaceae, Roseburia, Ruminococcus* and *Ruminococcus bromii.* Alteration in Bifidobacterium were recurrent in four of the studies with dietary fiber intervention and complex diets. Results from other RCTs are consistent with the studies included in this review indicating that soluble fibers including AXOS, GOS and RS may boost the presence of beneficial gut bacteria such as *Bifidobacterium, Ruminococcus*, and *Roseburia* (François et al., 2012; Kjølbæk et al., 2020). Furthermore, a study by Walton et al. demonstrating an increase in *Lactobacilli* after three weeks of AXOS intervention (Walton et al., 2012). These fibers are known to promote the growth of beneficial gut bacteria, particular *Bifidobacterium* and *Lactobacillus* (Davani-Davari et al., 2019; Xiao et al., 2020). *Bifidobacterium* members were among the earliest microorganisms that colonized the human gastrointestinal tract and are thought to provide health benefits to their hosts (O'Callaghan & van Sinderen, 2016). Increases in beneficial bacteria such as *Akkermansia, Bifidobacterium,* and *Lactobacillus* are often associated with improved gut barrier function and metabolic health (Dao et al., 2016; Dempsey & Corr, 2022; Hidalgo-Cantabrana et al., 2017; Krumbeck et al., 2018).

Out of five studies focusing on fiber intervention, two observed no significant changes in gut microbiota. The two reviewed studies by Canfora et al. found no alteration in gut hormones after inulin or beta-glucan interventions (Canfora et al., 2022). However, factors such as short duration may be a contributing factor for the lack of significant changes in gut microbiota (Canfora et al., 2022). The study also notes that shifts in fecal microbiota were highly individualized and tended to be fiber specific. The individual variability underscores the complexity in predicting the impact of dietary interventions on gut microbiota (Canfora et al., 2022). Existing research emphasizing that individual responses to dietary fiber can vary considerably based on baseline gut microbiota composition, genetics, and other factors (Fu et

al., 2022). The duration of fiber supplementation can play a significant role in observing changes in the gut microbiota (Fu et al., 2022). Longer interventions, like the 12-week trials by Müller et al. and Canfora et al. might be more effective in observing changes in gut microbiota, as shown in the results Table 1. However, various studies have shown that dietary fiber, including inulin, can modulate gut microbiota by promoting the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* (Cronin et al., 2021; Makki et al., 2018). This is also supported by a later RCT study from 2017 by Nicolucci et al. which found that prebiotic fibers containing oligofructose enriched inulin can significantly alerted gut microbiota composition in participant with overweight or obesity (Nicolucci et al., 2017).

The amount of fiber is also important to consider when looking at the results from the reviewed studies. A high dose of fiber might lead to more significant changes in gut microbiota (Cronin et al., 2021). For example, while 15 g/day of AXOS led to significant changes in microbiota composition (Muller et al., 2020), as did the same amount of GOS demonstrating an increase in *Bifidobacterium* abundance (Canfora et al., 2017). To support the findings from the reviewed studies, looking at a crossover trial from 2019 by Benítez-Páez et al. involving 30 overweight and obese individuals who had a BMI ranging from 25 to 40 kg/m². The intervention was a dietary modification trial where participants were given a wheat bran extract enriched AXOS. The amount of AXOS administered was 10.4 grams per day for four weeks. The results of this intervention were notable: there was an observed increase in the abundance of *Prevotella* in the gut microbiota of the participants after the AXOS intake (Benítez-Páez et al., 2019). These findings are relevant as they align with the observed outcomes in the current scoping review, emphasizing the potential for specific dietary fibers like AXOS to modulate gut microbiota composition.

Some fibers are rapidly fermentable, while others take a longer time (J. Slavin, 2013). This can influence the results observed, especially in short-term studies. Fiber is an extensive variety of compounds with various health effects (J. Slavin, 2013), and comparing different fiber quality and amounts may explain the inconsistency among the results on gut microbiota. The reviewed study by Zhang et al. experienced a decrease in several gut bacteria, including *Bacteroides, Blautia,* and *Eubacterium*, among others, after administration with 40g/d with RS, and *Ruminococcaceae* increased after RS intervention. The study only lasted for 4 weeks which is a short intervention (Zhang et al., 2019). The effects of RS on the gut microbiota

have been documented in other studies as well. For instance, resistant starch is known to be a prebiotic, which means it can serve as a food source for beneficial gut bacteria, such as some species of *Bifidobacterium* and *Lactobacillus* (Bendiks et al., 2022; McOrist et al., 2011).

The results from this scoping review indicate that interventions with fiber and complex diets have a greater effect on the gut microbiota than interventions with dietary supplements, as only one study demonstrated alteration in gut microbiota. Fiber-rich diets and complex dietary interventions typically involve a variety of foods that provide a wide spectrum of nutrients, including different types of fibers that can promote the growth of a diverse microbial community in the gut (Holscher, 2017). It is well established that diet is a major factor in shaping the composition of the gut microbiota. (David et al., 2014; Salonen et al., 2014; Walker et al., 2011). The reviewed study by Hansen et al. demonstrated that single components like gluten have the ability to alter gut microbiota (Hansen et al., 2018). Hansen demonstrated that numerous gut bacteria spices were significantly reduced after intake of a low gluten diet, among them the beneficial bacteria *Bifidobacterium* (Hansen et al., 2018). The findings from Hansen et al. are in line with an RCT study published by De Palma et al. in 2009, which found that adults with non-celiac gluten sensitivity who adhered to a gluten-free diet for a month experienced changes in gut microbiota, with a reduction in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* (De Palma et al., 2009). In contrast, a study from 2016 by Bonder et al. analyzing the gut microbiota in healthy adults following a low gluten diet for four weeks found no significant changes in gut microbiota compared to a control group (Bonder et al., 2016). The low-gluten diet´s effect on healthy adults may be due to changes in fiber intake. High gluten diet foods like wheat are rich in dietary fiber (Um et al., 2023). Taken together, these findings may suggest that following a gluten-free diet will decrease the intake of fiber-rich cereal foods, and a possible decrease in beneficial bacteria like *Bifidobacterium.* The reviewed study by Ren et al. demonstrated an almond-based low carbohydrate diet had the ability to change gut microbiota. *Eubacterium, Roseburia* and *Ruminococcus* were significantly increased (Ren et al., 2020). This is in line with an RCT study from 2018 by Holscher et al. that also demonstrated that almond consumption had the ability to alter gut microbiota. Almond consumption increased the relative abundances of *Lachnospira, Roseburia*, and *Dialister* (Holscher et al., 2018). The importance of diet in modifying the composition of the gut microbiota is becoming more widely recognized (Conlon & Bird, 2014). Numerous of studies have investigated the effect of dietary

intervention on gut microbiota (Johansson et al., 2013; Kovatcheva-Datchary et al., 2015; A. Nilsson et al., 2016; A. C. Nilsson et al., 2008). Diet could be assumed to be one of the most prominent factors influencing the microbiota composition (Moles & Otaegui, 2020). Nevertheless, despite numerous strategies proposed to modulate the human gut microbiota through dietary interventions, guidelines to achieve this goal have yet to be established (Moles & Otaegui, 2020). In general, a change toward greater diversity or richness in gut microbiota is considered to be beneficial (Lozupone et al., 2012).

In the reviewed study by Jones et al. investigating the effect on dietary supplements, no significant alterations in gut microbiota were found between the VSL#3 probiotics and placebo groups, despite the high dosage of probiotics administered to the probiotic group (Jones et al., 2018). This finding contradicts the common belief regarding probiotics' role in supporting gut health (Hill et al., 2014). Despite the findings by Jones et al. other studies suggest that probiotic supplementation can influence the secretion of gut microbiota. An RCT from 2015 found that an intervention involving VSL#3 probiotic (2 capsules per day) supplementation increased *Bifidobacterium* in healthy individuals between the ages of 65 and 85 (Valentini et al., 2015). However, the population in the study by Valentini et al., which focused on normal-weight individuals aged 65 to 85 years old, differed from that of the reviewed study by Jones et al., which examined obese adolescents aged 12-18 years old.
It should be recognized that the efficacy of probiotic supplementation is influenced by variables such as the specific strains and doses used, as well as individual factors like diet, genetics, and the existing gut microbiota composition (Hasan & Yang, 2019). These studies highlight that the impact of probiotics on gut microbiota can vary greatly depending on the population being studied. The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts provide a health benefit on the host” (Joint FAO WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, 2006). VSL#3, a probiotic combination containing eight live bacterial strains, has recently received a lot of attention for its combined effect (Cheng et al., 2020).

BMI varied considerably among the reviewed studies, with the lowest recorded being 18.6 kg/m^2 (Mano et al., 2018), and the highest between 28-40 kg/m^2 (Canfora et al., 2017). Typically, a BMI of 30 kg/m^2 is categorize as obese (World Health Organization, 2021). Studies have shown that diversity and richness of the gut microbiome are reduced in obese

subjects (Denou et al., 2016; Heiss & Olofsson, 2018). A study done on 61 healthy adults from Ukraine with a mean age 44.2 years found that *Firmicutes/Bacteroidetes* ratio rose with rising BMI (Koliada et al., 2017). These subjects were divided into four groups based on their BMI: those with a BMI of 18.5 kg/m2 (underweight), those with a BMI of 18.5 to 24.9 kg/m2 (normal), those with a BMI of 25.0 to 29.9 kg/m2 (overweight), and those with a BMI of 30.0 kg/m2 (obese) (Koliada et al., 2017).

In summary it was not possible to draw a conclusion from the reviewed studies regarding fiber, supplements, and complex diets on the effect on gut microbiota due to the heterogeneity among the included studies, including variation in study duration, type of fiber, supplements and diets utilized, metabolic health as well as differences in the populations being examined The responses of the gut microbiota to dietary interventions might vary depending on the type, amount, and duration of the intervention (Fu et al., 2022). BMI did also vary considereably among the reviewed studies thereby this might affect how the gut microbiota responded to dietary interventions. More research, particularly in the form of RCTs, is needed to demonstrate how characteristics such as genetics, age, sex, BMI, and baseline gut microbiota composition impact an individual's response to specific dietary modifications.

Impact on diet and dietary components on gut hormones

Interventions with dietary fiber, supplements and complex diets presented varied effects on gut hormones in the current scoping review. The reviewed study by Müller et al. documented a decrease in GLP-1 after AXOS intervention (Muller et al., 2020), whereas Zhang et al. Mano et al., and Ren et al. observed an increase in GLP-1 (Mano et al., 2018; Ren et al., 2020; Zhang et al., 2019).

GLP-1 is an incretin hormone that stimulates insulin secretion (Maselli & Camilleri, 2021). An increase in GLP-1, as seen after RS interventions by Zhang et al. may suggest that fiber has an effect on gut hormones. The reviewed study by Zhang et al. found that active GLP-1 levels increased significantly at 30 minutes during a meal tolerance test after 40g RS compared to control starch (Zhang et al., 2019). The early postprandial increase in GLP-1 suggest that RS might enhance early insulin secretion, which could potentially improve glycemic control (Zhang et al., 2019). This contradicts the RCT study by Al-Mana &

Robertson which measured GLP-1 every 30 minutes for 7 hours following RS intervention containing 48g for both breakfast and lunch, found that postprandial GLP-1 levels were significantly altered (Al-Mana & Robertson, 2018). However, results from studies on animals have demonstrated that fiber like RS can increase GLP-1 (Keenan et al., 2006; Zhou et al., 2008). Several studies have shown an association in increased intake of dietary fiber with enhanced control of body weight (Du et al., 2010; Howarth et al., 2006; Lattimer & Haub, 2010; J. L. Slavin, 2005). Fiber, especially soluble fiber, has been studied in various research contexts for its effects on gut hormones, like GLP-1 and PYY (Rebello et al., 2016).

The reviewed study by Ren et al. demonstrated an increase in GLP-1 in participants with type 2 diabetes after an almond based diet, in which the LCD group consumed 56 g/day of almonds, replacing 150 g/d staple food (Ren et al., 2020). This is in line with an RCT study from 2023 on overweight and obese adults, where they observed higher GLP-1 levels in those who consumed almonds compared to a placebo group (Carter et al., 2023). This underscores the need for broader research to determine almonds' effects on gut hormones like GLP-1 in type 2 diabetes patients. GLP-1 plays a significant role in regulating blood sugar levels and appetite, which could have potential implications for those with diabetes type 2 and for overweight and obese individuals (Maselli & Camilleri, 2021). It is also worth considering the demographic differences in the study populations. While the reviewed study by Ren et al. focused on participants with diabetes type 2, the study by Carter et al. included overweight and obese adults. It would be valuable to investigate if almonds have a uniform effect across diverse populations, or if these effects are modulated based on the health status or metabolic conditions of the individuals. Therefore, in type 2 diabetes, increased GLP-1 levels after dietary intervention may be beneficial due to its role in insulin secretion and having potential glucose-lowering effect (Hinnen, 2017). Understanding how different dietary components affect gut hormones is important since these hormones regulate appetite, glucose homeostasis, and energy balance (Chaudhri et al., 2006). Alterations in their blood concentration, therefore, influence obesity, diabetes, and other metabolic diseases (Lean & Malkova, 2016). Reviews have highlighted the benefits of low carbohydrate diets, including enhanced satiety, as well as the benefits for weight loss and metabolic parameters (Feinman et al., 2015; Noakes & Windt, 2017).

It was a consistent finding in this scoping review that PYY remained unchanged in most studies, except for the study by Hansen et al. which involved a low-gluten diet intervention, and the study by Genton et al. which involved an intervention with BCAAs (Genton et al., 2021; Hansen et al., 2018). PYY is a hormone that suppresses appetite (Karra et al., 2009). This may indicate that the dietary fibers, supplements, and complex diets have little effect on satiety signaling via PYY, or that the study duration or dosage were insufficient in the reviewed studies. Taken together, the fact that there are varied responses in GLP-1 and PYY to dietary fibers, supplements, and complex diets suggests that dietary composition can selectively influence the release of gut hormones. This might be important for designing dietary interventions for weight maintenance or diabetes management.

The reviewed study by Jones et al. found no significant impact on the gut hormones GLP-1, PYY, or CCK in obese adolescents after intervention with three sachets of VSL#3 probiotics for 16 weeks (Jones et al., 2018). This is in contrast with a 2014 RCT involving obese Italian children, where the intervention of two sachets of VSL#3 probiotics daily for four months demonstrated an increase in GLP-1 (Alisi et al., 2014). An animal study from 2013 found that intervention for 4 months with VSL#3 inhibited body weight growth and insulin resistance by altering the composition of the gut flora. VSL#3 increased the release of the hormone GLP-1, which resulted in less consumption of food and better glucose tolerance (Yadav et al., 2013). Despite existing studies, more research is needed to understand these effects and determine optimal use of probiotic supplements. Additionally, it would be helpful to explore the long-term effects of probiotic supplementation and their impact on obesity, and obesity related diseases. The use of probiotics as a part of obesity treatment is limited due to a lack of efficacy data and a lack of knowledge of their mechanisms of action (Nagpal et al., 2012).

Future research should include studies of longer duration to investigate if changes in GLP-1 and PYY are time-dependent. Further research should also investigate if the dose of the intervention food like dietary fiber, supplements and more complex diets will affect gut hormone levels. Metabolic status of individuals can greatly influence their response to dietary interventions, particularly those targeting gut hormone response (Zhao et al., 2017). The interventions included in this present scoping review have been performed in populations with different metabolic status. However, it is necessary to further explore the effect of different diets on metabolic regulation (e.g., normoglycemic vs. prediabetic etc.).

In summary, the diverse nature of the methodologies and findings in the reviewed research makes it challenging to reach a definitive consensus about the impact of fibers, supplements, and complex diets on gut hormones.

Impact of diet and dietary components on gut microbiota and gut hormones

Seven of the 13 studies in this current review demonstrated alterations in gut microbiota and gut hormones. Five of the studies did not report any change in gut microbiota or gut hormones, while one study reported only alterations in gut microbiota. However, it is important to note that the specific strains or types of gut bacteria assessed in these studies were not uniform. Additionally, the particular gut hormones that experienced changes were not consistent among the studies. This variation suggests a complex interaction between diet, gut bacteria, and gut hormones, indicating that different methodologies or subject populations can provide diverse results. Muller et al. reported that after an AXOS intervention, there was an increase in beneficial bacteria such as *Akkermansia, Bifidobacterium,* and *Lactobacillus* (Muller et al., 2020). Additionally, there was a decrease in GLP-1, a gut hormone involved in insulin secretion and appetite regulation (Muller et al., 2020). These findings suggest that AXOS intake can modify the gut microbiota in a manner that is thought to be beneficial, but the effect on gut hormones like GLP-1 can be more complex and potentially unexpected. It is important to consider methodological limitations, such as sample size, study design, and generalizability, which can affect the interpretation of the results. Zhang et al. found that interventions with RS led to a decrease in various gut bacteria, and an increase in GLP-1 (Zhang et al., 2019). This could indicate that certain dietary fibers can stimulate the secretion of GLP-1, even though the exact mechanisms and clinical significance of these microbiota changes need to be further explored. While a direct causal link cannot be established based solely on the study by Zhang et al. the findings hint that RS potentially having a direct or indirect influence on GLP-1 secretion, regardless of the alteration of the gut microbiota. The findings from the reviewed study by Zhang et al. may thus seem unexpected since several gut bacteria decreased, as RS is often associated with promoting a healthy gut microbiota (Bendiks et al., 2022). Genton et al. explored the effects of BCAAs and found that even though *Bifidobacterium dentium* and *Lacticaseibacillus paracasei* decreased, there were increases in the levels of GLP-1, PYY, and CCK (Genton et al., 2021), which are all important gut hormones for metabolism (Lean & Malkova, 2016). This indicates a possible

link between changes in the gut microbiota and hormonal responses that could affect energy metabolism and satiety. Ren et al. reported that after an almond intervention, there was an increase in *Eubacterium*, *Roseburia*, and *Ruminococcus*, along with an increase in GLP-1 (Ren et al., 2020). These bacteria are known to be involved in the production of SCFAs, which may play a role in the regulation of GLP-1 (Fusco et al., 2023).

The reviewed study by Hansen et al. found that a low-gluten diet led to significant changes in the composition of the gut microbiome, particularly a consistent decrease in the abundance of *Bifidobacterium species* (Hansen et al., 2018). The study also observed reductions in other important gut bacteria, such as *E. hallii*. Despite these significant microbial changes, the levels of GLP-1 remained unchanged following the low-gluten diet, while PYY was higher after a low gluten diet. While PYY levels responded to the low-gluten diet, GLP-1 levels did not, indicating that these hormones may be regulated by different mechanisms in response to diet, or that the changes in the diet composition.

The reviewed study by Johnstone et al. found an increase in the gut bacteria *Faecalibacterium prausnizii* and *Ruminococcus bromii* after an RS-WM diet compared to a weight loss diet (Johnstone et al., 2020). In this study, the gut hormones GLP-1 and PYY remained unchanged during WL diet, while ghrelin increased. GLP-1 and PYY are hormones associated with satiety (Karra et al., 2009; Meloni et al., 2013), and their stable levels might suggest that the RS-WM diet does not significantly alter the satiety signaling via these hormones, despite the changes in the microbiota. The increase in ghrelin, a hunger hormone, during the WL diet suggests that calorie restriction was perceived by the body, which responded by increasing the hunger signal (Johnstone et al., 2020). This is in line with an RCT study by Cummings et al. where 13 obese subjects underwent a weight loss diet for three months, found that ghrelin increased after weight loss (Cummings et al., 2002).

This current scoping review support the concept that dietary modifications can be a strategy to modulate gut hormone responses through changes in the gut microbiota. Over half of the summarized articles showed that diet and dietary factors can influence gut microbiota and gut hormones. Gut microbiota is known to influence various gut hormones, including GLP-1, CCK and PYY (Cani et al., 2009; Martin et al., 2019). However, drawing a direct line between specific bacterial species or groups and gut hormone levels is challenging due to the

heterogeneity among the reviewed studies. Further, there is probably multiple overlapping factors, such as the composition of the diet, the host's metabolism, and the individual's microbiota, contributing to the observed effects. Nonetheless, they do suggest that diets rich in certain fibers, prebiotics, and complex diets can affect both the gut microbiota and the secretion of gut hormones in ways that could be beneficial for metabolic health. Furthermore, whether these changes result in clinically relevant outcomes such as improved glucose control or weight management requires more investigation.

Limitations

The current findings should be interpreted with caution, as this review only includes 13 studies, which limits the ability to generalize the effect of dietary interventions on gut microbiota and gut hormones across different populations. The heterogeneity observed across the RCT studies could impose limitations on the findings of the current scoping review. Heterogeneity among the studies like variability in study designs, type of dietary fiber, supplements and diets, duration of the intervention, and different population among these studies could impact the interpretation of the results. Participant´s health status that was ranging from healthy, prediabetic, hemodialyse patients to obese adolescents which could contribute to the inconsistency in the results. Diverse populations may have varying baseline gut microbiota, which can be influenced by both genetic and environmental factors, including traditional dietary practices (Lozupone et al., 2012).

In over half of the reviewed studies on dietary fiber interventions, measurements of gut microbiota and gut hormones were assessed as secondary outcomes, and therefore these outcomes need to be interpreted with caution.

6.0 Conclusion

Based on the studies included in this scoping review different dietary interventions, such as dietary fiber, dietary supplements, and complex diets have the potential to change gut microbiota and satiety regulating gut hormones. The current scoping review points towards the fact that there is a heterogeneity of the studies, including the subjects, the dietary interventions, and the methods used for gut microbiota analyses. Furthermore, not all studies had gut microbiota or gut hormones as primary outcomes, which makes it challenging to derive solid conclusions about the effect of diet on gut microbiota and satiety regulation. Well-designed RCTs, with gut microbiota and gut hormones as primary outcomes are needed to fully understand the interaction between dietary interventions, gut microbiota, and gut hormones.

7.0 Future Perspectives

Understanding the relationship between diet, gut microbiota, and satiety hormones is important for developing targeted interventions to prevent or manage obesity and metabolic diseases. Gut microbiota may play an important role in energy metabolism and the gut-brain axis, but additional well-designed studies are needed to demonstrate the potential benefits in manipulating gut microbiota and gut hormones through diet. These varying effects in this scoping review highlight how individualized the response to dietary changes can be, pointing towards the need for more personalized nutrition strategies.

More randomized controlled studies specifically designed with gut microbiota and gut hormones as their primary outcomes are needed. Such studies would provide clearer insights into the interaction dynamics between dietary interventions, gut microbiota, and satiety regulation. Moreover, there's a clear necessity for standardized methodologies in terms of dietary intervention types, participant selection criteria, gut microbiota sampling, and outcome measurement techniques. This would enable more robust comparisons between studies and get a more comprehensive understanding of the field.

Long-term studies that follow individuals over time after dietary interventions would provide insights into the changes in gut microbiota and the long-term effects on satiety and weight management. As the field advances, there may be a move towards m personalized nutrition plans based on individual microbiota profiles and hormonal responses to optimize satiety and health outcomes.

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Appendix 1:

Search strategy: Medline:

Search Strategy:

#	Searches	Results
1	intestine flora.mp.	7
2	gut flora.mp.	2077
3	Gastrointestinal Microbiome/	33722
4	gut microbiota.mp.	34474
5	satiety.mp.	11432
6	Satiety Response/	2564
7	Satiation/	3822
8	Glucagon-Like Peptides/	1953
9	glucagon like peptide.mp.	18803
10	Peptide YY/	2308
11	PYY.mp.	2741
12	Ghrelin/	8206
13	ghrelin.mp.	12018
14	Hunger/	5915
15	gut hormon*.mp.	2522
16	Cholecystokinin/	10883
17	cholecystokinin.mp.	18803
18	CCK.mp.	26964
19	1 or 2 or 3 or 4	51771
20	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	82147
21	19 and 20	779
22	limit 21 to (humans and yr="2009 - 2022" and randomized controlled trial)	35
23	limit 22 to english	35

Search strategy: EMBASE

Database(s): **Embase** 1974 to 2022 September 29

Search Strategy:

#	Searches	Results
1	gut microbio*.mp.	53175
2	intestine flora/	82115
3	microbiome/	26424
4	gastrointestinal microbio*.mp.	2079
5	microbiota.mp.	92887
6	satiety/	15814
7	satiety.mp.	21270
8	hunger/	13216
9	satiety regulat*.mp.	123
10	glucagon like peptide/	1229
11	GLP-1.mp.	22928
12	peptide YY/	5192
13	PYY.mp.	3731
14	ghrelin/	17537
15	ghrelin.mp.	19594
16	appetite/	25079
17	gut hormon*.mp.	3603
18	satiety hormon*.mp.	612
19	cholecystokinin/	16578
20	cholecystokinin.mp.	32507
21	CCK.mp.	32837
22	1 or 2 or 3 or 4 or 5	143977
23	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21	136223
24	22 and 23	2098
25	limit 24 to (human and randomized controlled trial and yr="2009 - 2022")	107
26	limit 25 to english language	107

Appendix 2, CASP Evaluation

CASP Randomised Controlled Trial Standard Checklist:
11 questions to help you make sense of a randomised controlled trial (RCT)

Main issues for consideration: Several aspects need to be considered when appraising a randomised controlled trial:

- Is the basic study design valid for a randomised controlled trial? (Section A)
- Was the study methodologically sound? (Section B)
- What are the results? (Section C)
- Will the results help locally? (Section D)

The 11 questions in the checklist are designed to help you think about these aspects systematically.

How to use this appraisal tool: The first three questions (Section A) are screening questions about the validity of the basic study design and can be answered quickly. If, in light of your responses to Section A, you think the study design is valid, continue to Section B to assess whether the study was methodologically sound and if it is worth continuing with the appraisal by answering the remaining questions in Sections C and D.

Record 'Yes', 'No' or 'Can't tell' in response to the questions. Prompts below all but one of the questions highlight the issues it is important to consider. Record the reasons for your answers in the space provided. As CASP checklists were designed to be used as educational/teaching tools in a workshop setting, we do not recommend using a scoring system.

About CASP Checklists: The CASP RCT checklist was originally based on JAMA Users' guides to the medical literature 1994 (adapted from Guyatt GH, Sackett DL and Cook DJ), and piloted with healthcare practitioners. This version has been updated taking into account the CONSORT 2010 guideline (http://www.consort-statement.org/consort-2010, accessed 16 September 2020).

Citation: CASP recommends using the Harvard style, i.e., *Critical Appraisal Skills Programme (2021). CASP (insert name of checklist i.e. Randomised Controlled Trial) Checklist. [online] Available at: insert URL. Accessed: insert date accessed.*

Study and citation: Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Senility in Obese Prediabetic Individuals (Canfora et al., 2017).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	The study design is a double-blinded, placebo-controlled, parallel intervention over 12 weeks. Population 48 normoglycemic overweigh and obese healthy men and women. Population from Maastricht Netherlands.
	Intervention group receiving 15g/day of AXOS daily and the placebo group consuming a visually and taste-similar powder without any prebiotic fiber (i.e., maltodextrin).
	The primary aim was to assess the effect of Galacto-oligosaccharides (GOS) supplementation on peripheral insulin sensitivity in obese prediabetic individuals. Secondary outcome included microbiota composition and gut hormones GLP-1 and PYY.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ Randomization was carried out by an independent researcher and was stratified for sex and age. The allocation sequence was indeed concealed, reducing the risk of bias.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ Loss to follow up provided in a flow chart. 76 assessed for eligibility. 27 excluded due to that they don't meet the inclusion criteria, 1 declined, 1 used antibiotic, 1 other reason. According to the data all 46 was randomly assigned to either GOS or placebo were accounted for at the study`s conclusion. There were zero lost to follow up. The study did not mention that the study was stopped early.

Section B: Was the study methodologically sound?	
4. • *Were the participants 'blind' to intervention they were given?* • *Were the investigators 'blind' to the intervention they were giving to participants?* • *Were the people assessing/analysing outcome/s 'blinded'?*	Yes No Can't tell ☒ The participants and investigators were blinded. Does not mention if the people assessing/analyzing the outcomes were blinded. However, this study is a double-blinded trial, which means that neither the investigators administrating the interventions, nor the participants knew which treatment (GOS or placebo) they were receiving.

5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't tell ☒ Yes, this was set out in table 1. Participants baseline Characteristics. The groups were similar at the start at the trial.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ The study appears to have a clearly defined protocol, outlining the participant characteristics, intervention details, and the outcomes that will be measured. According to the study, aside from the primary intervention (GOS or placebo), both groups were treated the same. They were both asked to ingest their respective supplements (either GOS or isocaloric maltodextrin) three times per day with their regular meals for 12 weeks. Both groups also received the supplements in white powdered form in sachets and consumed them with a low-fat yogurt drink that did not contain probiotics or supplemented GOS. The follow-up, including experimental clinical investigation days (CIDs), dietary intake recording, and physical activity recording, appear to have been conducted in a parallel manner for both groups.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes No Can't tell ☒ Power calculation was undertaken. To detect a physiologically relevant difference in the change in peripheral insulin sensitivity of 20% with a standard deviation (SD) of 4, at least 17 participants per group were necessary to achieve 80% power and an alpha level of 0.05. Accounting for a projected 25% drop-out rate, the total planned recruitment was 46 participants. The results seem to be reported fairly comprehensively in terms of the outcomes measured and statistical tests used. P -values are reported for most outcomes.

8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes ☒ No Can't tell Confidence intervals were reported in a table.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes ☒ No Can't tell GOS supplementation led to a marked increase in the fecal abundance of Bifidobacterium species. This is consistent with prior research indicating that GOS can promote the growth of these beneficial bacteria.GLP-1 increased. No cost-effectiveness analysis were undertaken.

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study focused on prediabetic obese men and women between 45-70 years old. BMI: 28-40kg/m2. Difference in age, metabolic status, region, and other demographic factors could alter the outcomes. If the local population is similar to the study´s population depends on several factors. If the population is similar to the study`s population and if the outcomes measured are of interest, it could be relevant. However, if the foucs is with a broader metabolic or health outcomes, the studys results may not be directly applicable. Limitations: The study duration for 12 weeks, might not be long enough to see changes in some metabolic markers or health outcomes. Few participants included, it may therefore be appropriate to look for corresponding studies to obtain a more secure basis for drawing a conclusion about the effect of the measures.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes ☒ No Can't tell Benefit: The primary positive outcome from GOS supplementation was a marked increase in the fecal abundance of Bifidobacterium species. Limitations: Despite this increase, GOS supplementation did not lead to any significant changes in parameters like insulin sensitivity, energy metabolism, inflammatory profile, and other metabolic markers in the studied population, which were the primary endpoints. This implies that while GOS may support gut health by promoting beneficial bacteria, it may not immediately improve metabolic health markers in prediabetic overweight or obese individuals.

	Thus, before introducing GOS as an intervention, it's crucial to weigh its benefits against its costs and potential limitations, especially in comparison to existing interventions. Exploring the impacts of different dosages and forms of GOS supplementation might illuminate dose-response relationships and optimal dosages for desired effects which could be explored in new interventions.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key finding:
Significant increase in Bifidobacterium species (secondary outcome)
No significant changes in gut hormones GLP-1 and PYY (secondary outcome)

In summary, based on the information provided, the study appears to be well-designed and rigorous in its methodology. This study is thorough and appears to be well controlled. It provides insights into the specific impact of GOS supplementation on a prediabetic population with overweight or obesity.
The study examined the effects of 12-week GOS (galacto-oligosaccharide) supplementation on microbiota composition and functionality, primarily focusing on Bifidobacterium species. No significant changes in gut hormones.
GOS supplementation significantly increased the abundance of fecal Bifidobacterium species without affecting overall microbial diversity or richness. Based on this study alone, it might be premature to recommend GOS supplementation as a standalone intervention to improve metabolic or inflammatory markers in prediabetic overweight or obese individuals.
However, considering the variance in results among different populations from this and other studies, personalized approaches and more targeted research might be necessary before making recommendations.

Study and citation: Fiber mixture-specific effect on distal colonic fermentation and metabolic health in lean but not in prediabetic men (Canfora et al., 2022).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	Crossover RCT.
	Population: 45 participants from Maastricht Netherlands: Lean participants (BMI 20-24.9 kg/m²)
	Obese and prediabetic (BMI 25 -35.9 kg/m2)Age: 30-65 years old.
	The studies aimed to understand the effect on different fibers on SCFA production and their subsequent effects on metabolic parmaters in humans.The secondary outcome for this study were microbiota compostion.Two sudies, inulin study and beta glucan study.
	The research clearly identifies the population being studied; lean and prediabetic lean/ obese men. Population from Maastric Netherlands. Different fiber supplements are given as an intervention (long-chain inulin + resistant starch, INU, maltodextrin, beta glucan + RS, and BG). Maltodextrin (placebo used in both trials). Several outcomes are measured to assess the intervention`s impact, including breath hydrogen conditions, as well as fecal microbiota composition and SCFA.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ Randomization was performed by an independent researcher using permuted block randomization. Participants were allocated to intake of supplements in a random order. The study described that the study deign, was a randomized, placebo controlled.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ All the participants who entered the study were accounted for. Provided in a flow chart in the result section. Inulin study: one participants of the lean group was excluded from dietary intake analysis due to incomplete documentation of food intake. Beta glucan study: two participants from the lean group were excluded from dietary intake analysis due to incomplete documentation of food intake. The study was not stopped early.

x

Section B: Was the study methodologically sound?

4.	• Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blindeded	Yes No Can't tell ☒ Both the participants and investigators were blind to the treatment being administrated. This helps reducing bias in both administering the treatment and reporting outcomes.
5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't tell ☒ Baseline characteristics were clearly set out in Table 1 in the results section.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ Yes, there was a clearly defines study found in the design section. This ensure that each participant, irrespective of the group they belong to (fiber or placebo), follows the same procedures. It appears from the study that the researcher took considerable steps to ensure that both groups received the same level of care. The study mentions a Clinical Investigational Day (CID) and a 14-day washout period in-between CIDs. The washout period suggests the interval between different treatment phases in this crossover design, ensuring that any effects from a prior treatment have ceased. However, it does not specify any different follow-up intervals post-intervention for the groups. As a result, we can assume that the follow-up intervals were the same for both study groups.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?*	Yes No Can't tell ☒ Power calculations were undertaken based on the primary outcome. This was provided in the method section. The researchers based their sample size calculation on a previous study and used G power to estimate the required number of participants. They aimed to detect a 30% increase in circulating acetate concentrations, assuming a standard deviation of 5, to observe a physiologically relevant metabolic effect. They calculated those 9 participants per study group would be sufficient to detect this difference with 80% power at an alpha level of 0.05. To account for a potential dropout rate of 20%, they

• *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	recruited 12 lean and 12 overweight/obese prediabetic individuals for both studies. Primary and secondary outcomes were clearly stated. The studies aimed to understand the effect on different fibers on SCFA production and their subsequent effects on metabolic parmaters in humans.The secondary outcome for this study were microbiota compostion. Results were expressed clerly. Dropouts occurred in both study groups (prediabetic and lean) The study doesn't mention potential sources of bias or how these were managed. The statistical test were provided in detail under the method statistical analysis section. P-values are extensively reported for primary and secondary outcomes.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes ☒ No Can't tell Confidence intervals were reported in supplementary Table 1 a, b, c and d.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ Based on the data presented, it seems like the INU+RS fiber mixture might offer some metabolic benefits for lean individuals, though the benefits for overweight/obese prediabetic individuals are less clear. While no harms were reported, the study lacks a cost-effectiveness analysis so can't really tell if the benefits outweigh the harms and costs.

Section D: Will the results help locally?	
10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ If the patient population consist of a mix of lean and prediabetic overweight/obese individuals from 30-65 years old, this study may offer some insight and be relevant. However, the study is limited to men, so factors like gender, age, ethnic background, and other health conditions (beyond diabetes) could alter how applicable these results are to your population. The study does not appear to look at long-term impacts or include female participants. Only men were studies, outcomes could be different in women. The study mention limitaion like short duration og the intervention and the focus on male particpants. While acute effects were observed it is unclrear how these would be translated into long-term health benefits or risks. Primary outcome were gut hormones, and secondary outcomes were gut microbiota.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ The INU +RS combination appears to have promising effects on lean individuals, improving energy expenditure, carbohydrate oxidation, and postprandial insulin sensitivity. For this demographic, the intervention might provide greater value than existing dietary advice. However, the same cannot be concluded for overweight/ obese prediabetic individuals who did not show the same improvements. If introducing this intervention requires changes in diet, then time will be needed for education and monitoring. And also the cost of these specific fibers, as well as potential diagnostic tools. Healthcare providers would be trained on how to educate patients about the new fiber intake recommendations.

APPRAISAL SUMMARY: *Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organization? Could you judiciously implement this intervention without delay?*

Key findings:
No changes in gut microbiota (secondary outcome) and gut hormones (primary outcome)
The study offer valuable insight into how different types of fiber affect gut microbiota and gut hormones. The study compares different fiber mixtures, contributing to a nuanced understanding of their effects. However, due to its short-term nature and its focus on male participants only, more research is needed to confirm these findings and explore their applicability for broader populations. The inclusion of only male participants limits the generalizability of the findings to the female population, so applicability to a female population is uncertain. The short-term nature of the study limits understanding of the long-term impacts of such dietary interventions. The study was powered on the primary outcome, potentially affecting the reliability of findings on secondary outcomes which was gut microbiota. Additional studies, focusing on long-term interventions and including a more diverse participant pool, are essential to validate the findings and assess the real-world applicability. The observation of individual variability also highlights the need for personalized approaches in nutritional interventions. Until more comprehensive evidence is available, it would be premature to recommend changes to care/interventions based on this study alone.

Study and citation: Gut barrier and microbiota changes with glycine and branched-chain amino acid supplementation in chronic hemodialysis patients (Genton et al., 2021)

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes ☒ No Can't tell
	Randomized crossover design.
	Population: 27 hemodialysis patients from Switzerland, male and female. Age 61.2 ± 13.7
	Mean BMI 27.7 ± 5.1 kg/m2
	The study investigated the impact of amino acids (glycine and branched-chain amino acids (BCAAs), and then switched after a washout period. The primary outcome was evaluated by analyzing alterations in the compostion and diversity of faecal microbiota. Secondary outcomes include, gut hormones GLP-1, PYY and CCK, systemic inflammation, gut permeability, and fecal IgG. Various blood and fecal parameters were measured to evaluate these outcomes

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒ No Can't tell The statistician, blinded to the type of supplements assigned to each number, generated two lists of randomizations using Stata software. The randomization was done through the method of randomly permuted blocks with random block sizes of 2 and 4. It seems like the randomization was design well to eliminate systematic bias. Yes, the allocation sequence was concealed from investigator and participants.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒ No Can `tell Yes, this is shown in a flow chart. Figure 1 in the results section. There is no information from the provided text that that the study was stopped early.

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Section B: Was the study methodologically sound?

4.	• Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ The study was a double-blind study, which means neither the participants nor the investigators were aware of the which supplement (BCAA or glycine) the particpants were receving at any given time. The investigatros and the people who were assessing and analyzing the outcomes were also blinded to the intervention.
5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't ☒ Baseline characteristics were clearly set out in a table in Table 1 in the result section. At baseline, there were no differences in surrogate serum markers of systemic inflammation, intestinal permeability, as well as in appetite mediators, and endocannabinoids levels between both groups as shown in table 1.The differences of these parameters between Months 4 and 0 of each supplementation, independently whether they started or ended with BCAA or glycine, are shown in Table 2.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ There was a clearly defined study protocol. Primary intervention was the administration of BCAA or glycine supplementation. Yes, the follow-up intervals appear consistent. Outcomes were measured at the start and end of each supplementation period.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?*	Yes No Can't tell ☒ The power calculation was performed for both the primary outcome, which is gut microbiota composition, and secondary outcomes, utilizing available data from the literature for these calculations. The highest N (sample size) was reached when performing the power calculation for gut microbiota composition. Multiple outcomes were measured. Results are expressed in median values. The results focus on baseline differences and then difference between months 4 and 0 for each supplementation. One patient did not complete the

• *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	baseline tests nor start the supplementation. Nine patients did not complete the study. It was a differential drop-out. 27 out of the initial 37 randomized patients completed the study. The Wilcoxon rank-sum test was used for the data in Table 1. Multiple mixed linear regression models were used for IL-6, GLP-1, CCK, and PYY. PERMANOVA was used for microbiota similarity analysis. P-values were reported.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes No Can't tell ☒ CIs is reported in a supplemental table 2.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ The study does not explicitly mention any adverse effects or harms caused by the interventions. However, the decrease in L. paracasei and Bifidobacterium with BCAA supplementation might be seen as an unintended effect since these are considered beneficial bacteria. Given the limited changes observed and the absence of information on adverse effects and cost-effectiveness, it is challenging to conclusively determine whether the benefits of the experimental intervention outweigh the harms and costs based only on the provided information. Further details and additional studies would be necessary for a comprehensive assessment.

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study looked at various serum markers of systemic inflammation, intestinal permieability, appetite mediators. Nine pateints did not complete the study, which may itroduce some level of bias. The study lacked a control group, which makes it challenging to determine if observed effects (or the lack of them) were due to the intervention or other factors. This is a significant limitation if one wants to apply the results to a broader population confidently. The study was conducted in a specific geographic region (Switzerland), and results might differ in a different environment or cultural context. The potential decrease of beneficial bacteria with BCAAs supplementation might be a concern. If the local population already has a decreased abundance of these bacteria due to other factors (e.g., local diet or prevalent use of antibiotics), introducing BCAAs could exacerbate that.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ Resources Needed: Time: Based on the study, the duration of supplementation is significant but doesn't indicate that extensive time is required for administration. Finances: The study doesn't specify the financial cost of glycine and BCAAs supplementation, but this should be considered when weighing potential benefits. Skills Development/Training: The administration of amino acid supplements is relatively straightforward, but any specific protocols should be considered BCAAs supplementation led to a decrease in the abundance of the beneficial bacteria Bifidobacterium dentium and Lacticaseibacillus paracasei.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key findings:

Neither glycine nor BCAAs supplementation significantly impacted serum levels of cytokines, appetite mediators, intestinal permeability markers, or fecal IgA. BCAAs supplementation, however, reduced the abundance of beneficial bacteria L. paracasei and B. dentium. Impact on gut microbiota primary outcomes, and gut hormones secondary outcomes.

The study faced several limitations, including the absence of a control group, and a small sample size. While the study provides insights into the potential effects of glycine and BCAAs supplementation in haemodialysis patients, the lack of significant impact on several key parameters and the presence of various study limitations necessitate caution. The observed reduction in beneficial bacteria due to BCAAs supplementation and the unclear clinical significance of increased fat-free mass with glycine highlight the complexity of implementing such interventions without a thorough understanding of their mechanisms and effects. Given the current evidence, it would be premature to recommend immediate changes to practice or care interventions solely based on this study.

Study and citation: Moringa Olifera Leaf Supplementation as Glycemic Control Strategy in Subjects with Prediabetes (Gómez-Martínez et al., 2021).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	Double-blind parallel trial conducted.
	Population: 65 prediabetes participants from Spain, age 40-70. BMI: 35 kg/m2
	Intervention six capsules of dry MO leaf powder daily for 12 weeks. Placebo given (microcrystalline cellulose).
	Primary outcome: FBG and HBA1c. Secondary outcome: insulin, gut hormones, and analysis of human gut microbiota.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ Participant were randomized into a simple block randomization of 1:1. To ensure allocation concealment containers were labelled A or B and only one person (non-researcher), supplied the corresponding containers to the participants once the intervention had been assigned using the randomization sequence
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ A detailed flow chart was provided in Figure 1. The study kept trach of all participants from the start to the conclusion. 5 dropouts (less then 20%) No mention of the study being stopped early.

Section B: Was the study methodologically sound?	
4. • Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analyzing outcome/s 'blinded'?	Yes No Can't tell ☒ The participants were blind to the intervention given. Double-blind randomized trial. The investigator and the people assessing /analyzing outcomes were blind to the intervention.

5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't ☒ The baseline characteristics of each study group were clearly set out in Table 1 in the results section. No differences were observed in the sex proportions, age or anthropometrical values between the subjects in the MO and PLC groups. The intervention did not modify the anthropometrical measures of the subjects in any of the groups
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ A clearly defined protocol. Both study groups received the same level of care. They were treated equally in terms of the study protocol, additional interventions, and follow-up intervals.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes No Can't tell ☒ Does not find any information about whether a power calculation was undertaken in the study. Several outcomes were measured and specified. Statistical method used: Independent samples T-test and Chi-square test, ANCOVA, GLM. P-value are consistently reported alongside results.
8.	Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes No Can't ☒ Yes, CIs reported in a table 4 (gut microbiota) , 5 (gut hormones) and 6 in the result section.
9.	Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?*	Yes No Can't tell ☒ Whether the benefits outweigh the harms and costs would depends on the actual costs of the intervention. Glycemic Control: Subjects who consumed MO for 12 weeks showed a decrease in

• *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	both Fasting Blood Glucose (FBG) and HbA1c levels, whereas the placebo group showed an increase. 58% of subjects in the MO group saw improved HbA1c levels compared to 38% in the placebo group. A detailed cost-effectiveness analysis is not provided in this study. MO consumption did not show any harmful effects liver or kidney function. Overall, the study suggests that MO supplementation might provide benefits in glycemic control for individuals with prediabetes. However, to fully determine if the benefits outweigh the harms and costs, more extensive studies, longer intervention durations, varied dosages, and a comprehensive cost-effectiveness analysis would be beneficial. MO did not cause any significant adverse effects on hepatic or renal functions in the studied dosage. But it is important to study long term effects in a broader context.

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The primary outcomes of the study were HbA1c and fasting blood glucose levels. If the local population is primarily concerned with these outcomes, then the results would be relevant. The authors acknowledge several limitations, including the variability of individual results, the moderately raised mean glycaemia before the intervention which might have reduced the observed effects, and the exploratory nature of some analyses. They also note that this study was more robust than some previous interventions due to its blind design and appropriate comparison groups. .
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ While MO showed potential benefits in glycemic control, the evidence from this study might not be strong enough to warrant its widespread adoption as a primary intervention for prediabetes. Starting with MO supplementation may not require much time and input, but it may require some training and information about the supplement. Healthcare professionals may require education about the product and dosage. Financial aspect is minimal.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key findings:

Gut Microbiota:No significant differences in rates of change between PLC and MO groups for most of the studied microbiota, except for Enterococcus spp., which showed no significant differences between groups from 0 to 12 weeks.
Gut Hormones:
Ghrelin, PYY, and GLP-1 levels showed no significant between-group differences from 0 to 12 weeks

The limitations of the study, small sample size, the variability of individual results, the moderately raised mean glycaemia before the intervention which might have reduced the observed effects.

Study and citation: A low gluten diet induces changes in the intestinal microbiome of healthy Danish adults (Hansen et al., 2018).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	The study was designed to evaluate the outcome of two different dietary interventions, a low gluten diet and high gluten diet. A crossover study.
	The primary outcome was the alteration in gut microbiota composition. Secondary outcome were blood markers such as gut hormones like GLP-1 and PYY.
	Population: 60 healthy Danish adult 22-65, who did not suffer from coeliac disease or other gastrointestinal disease. BMI: 25-35 kg/m2.
	Participants underwent two dietary interventions: one with low gluten intake (~2g/day) and one with high gluten intake (~20g/day). The interventions were provided for 8 weeks each, separated by a washout period. After one dietary intervention, they switched to other.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ Randomization was carried out using a website, www.randomization.com, by an investigator who had no contact with the participants. The use of a dedicated randomization website and the involvement of an independent investigator suggests the randomization was done appropriately. The allocation sequence was generated by an independent investigator who had no contact with the participants.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ This was provided in a flow chart, in the supplementary figures. No, lost to follow up. All the 60 participants who entered the study were accounted for. They study was not stopped early

Section B: Was the study methodologically sound?

4. • Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ The participants and investigators were blinded until the first examination day. However, the blinding was not maintained after that, not possibly because the nature of the diet (low-gluten vs. high-gluten) would be easily recognizable by the participants. It is uncertain whether this affects the outcome.
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Where there any difference between the study group that could affect the outcomes/s?*	Yes No Can't tell ☒ Baseline characteristics were cleary set out. This were cleary set out in a table (supplementary tables). Some particpants were lean, while other were overweight or obese. Men and women. The characteristics of the patricipants were well balanced between the groups.

6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ Study protocol clerary defines. During the two dietary interventions, participants were provided specific low-gluten or high-gluten products. There is no indication that other tests or treatments were given that were different between the study groups. Any additional tests, treatments, or measurements applied to one group seem to have been applied to the other group as well. The participants were followed up with a telephone call every second week during both interventions, which suggests that follow-up intervals were the same for both groups. Dietary compliance was monitored through a study diary, bi-weekly follow-up calls.

Section C: What are the results?	
7. Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒ No Can't tell Power Calculation: Estimations were based on 85% statistical power to detect a difference of 0.4 standard deviation in metabolic quantitative traits, based on previous observations from the MetaHit study. It was estimated that 51 individuals were needed, but to allow for a 15% dropout after randomization, a total of 60 participants were invited for participation. Additionally, based on observed standard deviations for the MGSs changing during the low-gluten and high-gluten interventions, we concluded that the number of included subjects was adequate to provide evidence of a changed intestinal microbiome after a low-gluten diet compared with a high-gluten diet. Outcomes Measured and Specification: The outcomes measured were clearly specified and include changes in the composition and function of the intestinal microbiome, urine metabolome, and various measures of host physiology. Specific measures such as breath hydrogen concentrations, postprandial well-being, and specific bacterial abundances were assessed. Expression of Results: The results were expressed both in narrative form and through figures and tables, providing detailed statistical data. However, the type of outcomes were not binary, so relative, and absolute effects in this context were not applicable. Results for Each Outcome at Each Follow-Up Interval: The study reports results for different outcomes. Missing or Incomplete Data: The study reports that 60 individuals were included, and 54 participants had more than two visits and were included in the analyses, suggesting some data may have been missing or incomplete, but it does not specify the nature or extent of this missing data. Differential Drop-Out: Yes, there was differential drop-out. Originally 60 participants were included, but only 51 completed the study, which could affect results. Potential Sources of Bias Identified: The study does not explicitly discuss potential sources of bias. Statistical Tests Used: Several statistical tests were used, including linear mixed model, paired t-test, and false-discovery rate (FDR). These were used to compare differences between the two diet regimens and to identify changes in bacterial abundance and other measures. P-value reported, indicating significance level.

8.	Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes ☒ No Can't tell CI were reported in supplementary Table 2.
9.	Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ The study indicates that an 8-week low-gluten diet intervention in healthy middle-aged adults led to several changes, although it doesn't fully explore whether these changes are beneficial or harmful in the long term. Additionally, the study does not discuss costs, making it challenging to answer whether the benefits of the experimental intervention outweigh the harms and costs. No health implication was reported. Most physicians are not trained in nutrition therapy, which makes it difficult to counsel patients. Furthermore, discussing nutrition with patients takes time.

Section D: Will the results help locally?

10.	Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study was conducted on 60 Caucasian Danish adults without coeliac disease, diabetes, or any other self-known disorders, aged between 22 and 65 years old, who were healthy by physical and biochemical examination, weight stable, and had a BMI of 25–35 kg/m2 and/or increased waist circumference. Since the study involves apparently healthy middle-aged adults and may not be generalizable to other age groups or individuals with specific health conditions. If gluten intake is a significant concern in the local population, and there is an interest in understanding its impact on gut microbiome, the study outcomes may be important. For this study primary outcome were gut microbiota, and secondary outcome were gut hormones. The reduction in certain beneficial bacterial species like Bifidobacterium is noteworthy, especially considering its importance in gut health. The study observed changes in plasma PYY, suggesting possible impacts on satiety. While this could have potential implications for satiety, it's not directly addressed in this study. The study is conducted over 8 weeks, so the long-term effects of a low-gluten diet on gut microbiota are unknown. Additionally, the study implies that effects might be more due to

	changes in dietary fibers than gluten itself. If you are interested in the effects of gluten specifically, this might complicate the findings for your context. Limitations: Ensuring strict adherence to dietary regimens and accurate self-reporting of food intake can be challenging. There might be variations in dietary fibers from non-gluten sources, which can influence the results.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ It appears that a low-gluten diet induces certain alterations in the gut microbiome composition and fermentation, which are not observed under a high-gluten diet. The low-gluten diet was associated with a significant weight loss. These changes were accompanied by a reduction in body weight and alterations in markers related to intestinal fermentation, without any observed adverse health implications in the short term. However, the long-term health consequences remain unknown. The value of the low-gluten intervention would be particularly dependent on individual health outcomes, cost-effectiveness, and the adaptability of the intervention to different populations and health conditions. Given the specific changes observed in gut microbiota and the potential for weight loss, the experimental intervention may be of value for certain populations.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key Findings:
Gut Microbiome Composition: The primary trial endpoint changed with the low-gluten diet, particularly impacting the gut microbiome composition and functional potential. A significant change was observed in the relative abundance of 14 bacterial species, with the Bifidobacterium species showing a consistent decline.
The study observed an increase in PYY.

Some limitations:
Ensuring strict adherence to dietary regimens and accurate self-reporting of food intake can be challenging.
There might be variations in dietary fibers from non-gluten sources, which can influence the results.

Study and citation: Probiotic Supplementation Increases Obesity with No Detectable Effects on Live Fat or Gut Microbiota in Obese Hispanic Adolescents; a 16-week, randomized placebo-controlled trial (Jones et al., 2018).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes ☒ No Can't tell
	The study was designed as a 16-week parallel, double-blind, and placebo-controlled trial to assess the outcomes of probiotic supplementation (VSL#3) versus a placebo in obese
	Population: obese Hispanic adolescents aged 12–18 years who met the set inclusion and exclusion criteria. BMI percentile ≥95th for age and gender.
	The primary outcomes were changes in gut microbiota and gut-derived appetite regulating hormones. Secondary outcomes included body composition, liver fat and liver fibrosis, plasma levels of insulin and glucose, and food intake. All outcomes were clearly defined and measured.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒ No Can't tell Participants were randomly assigned to either the treatment (probiotic) or control (placebo) group. Randomization was carried out using an "adaptive stratified block design" to ensure a balanced distribution of sex between the two groups. The adaptive stratified block design, coupled with the use of a random number generator, should be effective in eliminating systematic bias related to the variables they stratified for (in this case, gender). The process involved a designated statistician and a member of the research team (both not directly involved in the study) overseeing the randomization. They distributed unmarked packets to a blinded study team member who then delivered these packets to the research participants. Furthermore, the principal investigator and staff of the Diabetes and Obesity Research Institute were blinded to the intervention group of each participant until the study concluded.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒ No Can `tell Presented in a flow chart, figure 1. Lost to follow up was 0.

x

Section B: Was the study methodologically sound?

4. • Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ Yes, the participant and the investigators were blind to the intervention they received. Does not specify if the individuals assessing or analyzing the outcomes were blinded.
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes No Can't tell ☒ Baseline characterics were cleary set out in supplementary table 1. The study groups were similar at the start of the RCT. Independent t-tests confirmed that both groups were similar for age, weight, height, body composition, liver fat, liver fibrosis, fasting blood levels, and self-reported energy and macronutrient intake before beginning the intervention. Notably, the placebo group had a higher baseline BMI than the probiotic group. The study mentions that they utilized an adaptive stratified block desing to ensure a sex balance between the VSL#3 and placebo groups. This indicates that they made efforts to esnure that there was a balance in gender bewtween the two groups..
6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ There is a clearly defined protocol. The study appears to have taken multiple steps to ensure that the groups were treated equally. Both groups received the same procedures and assessments: medical exams, stool samples, fasting blood draws, MRI scans, dietary recalls, etc. The difference between the groups was the type of supplement they received (active probiotic culture vs. inactive placebo.

Section C: What are the results?

7. Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?*	Yes No Can't tell ☒ No mention of power calculation. Multiple outcomes were measured, including changes in gut microbiota, and gut hormones. Some participants were excluded from the study due for various reasons (e.g., antibiotic use etc.). Reducing final sample size n=19 from initially randomized 25. One withdraw before completing the intervention. The study acknowledges some sources of potential bias. For instance, they noticed a significant

• *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	difference in baseline BMI between the placebo and probiotic groups. Linear mixed models, independent t-tests, chi-square tests. P-values were reported for many of the outcomes.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes ☒ No Can't tell CI reported in a Table 2 in result section.
9. **Do the benefits of the experimental intervention outweigh the harms and costs?** *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No ☒ Can't tell The intervention had no significant effect on the overall composition of the gut microbes, or gut hormones. The participants in the probiotic group showed a significant increase in adiposity (fat percentage). Does not provide a cost-effectiveness analysis in this study. The benefits of the probiotic seem limited since the probiotic did not show any positive changes in gut microbiota or gut hormones. Based on the information provided, it is challenging to definitively conclude whether the benefits of the experimental intervention outweigh the harms and costs, primarily due to the increased adiposity observed and the absence of cost-effectiveness analysis

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?*	Yes No Can't tell ☒ The study focused on adolescents. Notably, the placebo group had a higher baseline BMI than the probiotic group. The outcomes consideres in the study realated to gut microbiota and gut hormones. If the local poplation is interested in these outcomes, then the study is relevant. For this study the primary outcomes were changes in gut microbiota and gut-derived appetite regulating hormones Several participants were excluded for various reasons like antibiotic-use, probable diabetes, etc. This may affect the generalizability of the results, especially if your local population has a higher prevalence of such exclusions.

• *Are there any limitations of the study that would affect your decision?*	The sample size is relatively small, which may limit the generalizability of the findings. There were baseline differences in the gut microbiota of the two groups, which might affect the outcomes. Future studies might need to ensure better matching or stratification during randomization. Limitations: Small sample size. Since no prior studies examined the impact of VSL#3® on the gut microbiota of youth, there was limited data to perform a power analysis for changes in the gut microbiota, which might have affected the study's outcomes. Using self-reported dietary recalls could introduce bias, as participants may not remember or may underreport/overreport their intake. This limitation could have affected the study's ability to detect small changes in energy intake that may have contributed to increased adiposity during the intervention
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ The probiotic intervention did not significantly alter gut microbial composition, gut hormones, or fasting blood measures compared to the placebo. To integrate intervention of VSL#3 training of health care professional may be needed. VSL#3 is expensive, so may be affordable compared to other interventions. There were no adverse effect of the intervention, except, the participants in the probiotic group showed a significant increase in adiposity (fat percentage), while the placebo group exhibited a decrease

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key findings:
The probiotic intervention did not significantly alter gut microbial composition, gut hormones, or fasting blood measures compared to the placebo.
Probiotic supplementation was associated with an increase in adiposity, while the placebo group exhibited a decrease.
Limitations:
The sample size is relatively small, which may limit the generalizability of the findings.
There were baseline differences in the gut microbiota of the two groups, which might affect the outcomes
The observed increase in adiposity in the probiotic group warrants further investigation.
The small sample size limits the statistical power to detect changes in gut microbiota.
The use of beverages with artificial sweeteners as a delivery mechanism for the probiotic/placebo might have affected the gut microbiota, though the exact effects of these sweeteners on the microbiota remain unclear.

Study and citation: Nondigestible Carbohydrates Affect Metabolic Health and Gut Microbiota in Overweight Adults after Weight Loss (Johnstone et al., 2020).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes ☒ No Can't tell
	
	Crossover RCT.
	Population: 19 volunteers from London with a BMI of 27-42 kg/m² participated in the study
	The study's primary aim is to examine the effects of nondigestible carbohydrates, particularly resistant starch type 3 (RS-WM), on weight maintenance (WM) after a weight loss (WL) period. Secondary outcomes include changes in fecal microbiota composition, microbial metabolite concentrations, and gut hormones. Out of 40 initially screened, 24 were recruited and 19 volunteers with a BMI of 27-42 kg/m² participated in the study.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒ No Can't tell Participants were randomly assigned to a diet. Computer-generated random numbers were used to assign the subjects. However, the specific method of randomization (such as block randomization, stratified randomization, etc.) is not detailed in the study.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒ No Can `tell Shown in flow chart in Figure 1 in the methods section. Out of 40 initially screened, 24 were recruited and 19 volunteers with a BMI of 27-42 kg/m² participated in the study. The study was not stopped early.

Section B: Was the study methodologically sound?	
4. • Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded' •	Yes No Can't tell ☒ It doesn't provide explicit information on whether the study employed blinding at different stages. A crossover study so the invetigators and people assessing the study are most likely not blinded. It is uncertain whether this affects the outcome.

5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes ☒ No Can't tell This was cleary set out in a Table 1 under the result section. There are apparent differences between males and females in these characteristics, which is expected due to physiological differences between the sexes.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☒ No Can't tell Yes, there is a clearly defined protocol. Based in the information provided, the study appears to be well-defined and covers a range of aspects, including the types of diets administrated, the duration of each dietary phase, how often participants are to be assessed, and what kind of measurement will be taken. The study does not specify any additional interventions or treatments outside of the experimental diets. The study mentions that all participants attended 3 times per week for body-weight assessment and food provision.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒ No Can't tell Power calculation was undertaken. The information provided indicates that a sample size of 20 subjects has approximately 90% power to detect differences, using a 2-sided test at an alpha level of 0.05, assuming an effect size (Cohen's d) of 1.0. Various outcomes were measured including energy intake, body weight, body mass index (BMI), fat mass, fat-free mass, blood pressure, and other variables. These are clearly specified. Results are presented as means ± standard deviations (SDs) and sometimes include the standard error of the difference between the means (SED). The study seems to focus on continuous variables. Results are reported in tables that present outcomes across different dietary protocols at multiple times. However, it is not entirely clear how many follow-up intervals were included. Does not mention missing or incomplete data. P-values are reported Potential sources of bias are reported
8.	Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes ☒ No Can't tell CI reported in a table in table 4 and 5 in the result section.
9.	Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:*	Yes ☒ No Can't tell RS promoted the growth of specific beneficial bacteria like R. bromii and Eubacterium rectale, both

• *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	of which are known for their roles in fermenting resistant starch and promoting gut health. RS did not significantly affect hunger or satiety hormones, which suggests it may not aid in weight loss through appetite control. The study does not seem to include a cost-effectiveness analysis. Based on the study, RS supplementation appears to have several benefits, particularly in promoting a healthy gut microbiome and better metabolic health. However, it does not significantly alter SCFA concentrations or affect hunger and satiety, and its effects may vary between individuals. No harms reported

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study recruited volunteers with BMI raning from 27-42, excluding those with medical conditions or medications- These need to be compared with the target population. To determine the applicability of this study to a local population or context, one would need to closely analyze the similarities and differences between the study participants and the target population, assess the relevance and importance of the study outcomes, consider any additional outcomes of interest, and weigh the study's limitations. Need to assess whether changes in these specific bacterial species are relevant to the population's health, given the known functions and effects of these bacteria. No significant changes in GLP-1 levels after RS consumption. Relevance: Consider whether the modulation of gut hormones is a key concern for this population, especially in relation to metabolic health and satiety. The short duration of the study is a limitation; longer-term studies might reveal more about the sustained impact on gut microbiota and hormones.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ Adopting a new diet intervention might require education for both patients and staff. Additionally, there might be expenses linked to educational materials, training sessions, and potential monitoring equipment or tests to track outcomes. Given the study's findings and its limitations, it might be premature to implement changes based solely on this paper.

APPRAISAL SUMMARY: *Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?*

Key findings:

The RS-WM diet led to a significant increase in Ruminococcus bromii, and a significant increase in the percentage of Faecalibacterium prausnitzii was also observed after the RS-WM diet, compared to WL-diet.

Regarding gut hormones the researchers found that ghrelin increased significantly after the WL diet period relative to the WM containing RS diet and WM diet. There were no significant changes in the gut hormones GLP-1 and PYY levels.

In conclusion, based on the provided methods, the study appears well-constructed, detailed, and rigorous. The paper offers valuable insights into the interaction between diet, specifically RS, and gut microbiota, showcasing the potential metabolic benefits of RS. However, due to its limitations in study duration, sample size, and generalizability, the results should be interpreted with caution.

Study and citation: Riboflavin Supplementation Promotes Butyrate of Gross Compositional Changes in the Gut Microbiota (Liu et al., 2022).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	A randomized, placebo-controlled, double-blind, parallel-group trial that investigated the effects of riboflavin supplementation on butyrate production and gut microbiota composition.
	Population: 105 males and females from the Netherlands, healthy weight range, 18-60 years old.
	Intervention clearly defined.
	Primary outcome were the effect of riboflavin supplementation on Faecalibacterium prausnitzii abundance. Secondary outcome included the microbiota composition and gut hormones
	Participants who were randomized into three groups: one group receiving a daily dosage of 50 mg of riboflavin, another group receiving a daily dosage of 100 mg of riboflavin, and a placebo group
	The study have a comparator in form of a placebo group
	Clearly defined outcomes

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ The study states that the participants were randomly assigned into three groups but does not elaborate on the method used for randomization.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ Provided in a flow chart in Figure 1 in the result section. Of the 157 volunteers who gave informed consent, 52 were excluded because of either health-related issue, body mass index (BMI) above the defined cutoff, or study dropout.

x

Section B: Was the study methodologically sound?

4.	• Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ The participants and investigators were blind to the intervention they were given. Not explicity mentioned for the people assessing, but in double blinded studies we generally assume that people assesing outcomes are also blind to the treatment
5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't tell ☒ The study does a good job of presenting these in a tabular form to allow for easy comparison. It appears that there were no statistically significant differences among the groups in these characteristics. Characteristic are provided in in table 1 in the result section. Differences between groups were tested with independent samples using the ANOVA test or the Kruskal–Wallis test for non-normally distributed continuous variables (or the Mann–Whitney *U* test in case of unfulfilled test assumptions.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ The study seems to be well-designed with a clearly defined protocol. Participants were randomly assigned into three groups and the study was registered on ClinicalTrials.gov. It also underwent approval by an institutional review board, suggesting that it adheres to ethical guidelines. It appears that apart from the experimental riboflavin intervention, each group received the same level of care and was subjected to the same data collection methods and timing.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?*	Yes No Can't tell ☒ Power calculation is undertaken. The researchers performed a power calculation to determine the appropriate sample size for their study, ensuring it has enough power to detect a statistically significant effect if one exists. The outcomes measured are clearly specified. The results are expressed through means, percentages, p-values, and various statistical tests. For binary outcomes, there is no explicit mention of relative and absolute effects being reported.

• *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	It seems that results were comprehensively reported for each group at different time points, including baseline measures and outcomes following riboflavin supplementation. Various statistical tests like ANOVA, Kruskal–Wallis test, Wilcoxon signed-rank test, and Mann–Whitney U test were employed. Two-sided p-values <0.05 were considered statistically significant. P-values were reported, and statistical significance was defined as $p < 0.05$.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes No Can't tell ☒ CI were reported in a supplementary Table 1.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ The study does not explicitly include a cost-effectiveness analysis. The primary objective of the study, which was an increase in the amount of F. prausnitzii, was not reached. This indicates that the intervention did not achieve its primary goal. But there were no harms reported. However, there were some positive outcomes reported such as increased butyrate levels in the riboflavin combination group and enhanced bacterial network after riboflavin intervention. Moreover, a trend of increased insulin and GLP-1 concentration was observed in the 100 mg/d group. Overall, while the primary objective was not achieved, the intervention had some effect on the gut microbiota, particularly its activity. The intervention appears to have a low risk and minimal resources requirements.

Section D: Will the results help locally?

10.	Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study recruited healthy adults from the Netherlands, with both male and female participants, and a BMI ranging from 18-25 kg/m2. The duration was short (2 weeks), which might not have been suffiecent to see changes in gut microbitoa composition. Participants in the study were already healthy, with an optimal microbiota composition.This might have reduced the chances of seeing significant changes. The study mentions that a standardized mixed meal test might have been more appropriate for investigating effects on appetite. It might be beneficial to know the long-term effects of riboflavin supplementation before applying the results to the local population.
11.	Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ Introducing riboflavin supplementation as an intervention to enhance gut microbiota activity, specifically increasing butyrate production, seems promising in this study. But the goal to increase Faecalibacterium prausnitzii was however not reach. The intervention appears to have a good safety profile and requires minimal resources. However, the final decision would depend on a thorough assessment of current interventions, patient needs, and available resources. Another important factor would be to determine the duration and potential long-term benefits of the riboflavin supplementation, as the study had a limited 2-week intervention period.

APPRAISAL SUMMARY: *Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?*

Key findings.
The study did not meet its primary outcome increasing F.prausnitizii levels. While there were no significant changes in gut microbiota composition, there was an increase in butyrate concentration. No significant changes in GLP-1 .
The study seems to be well-designed with a clearly defined protocol.
The study appears to have been methodologically rigorous, with careful consideration given to sample selection, data collection, and confounding factors. Limitations: Small sample size, and was conducted over a short period (3 months)

While the primary goal wasn't achieved, the intervention showed some positive effects with a good safety profile and minimal resource requirements. Considering the application in a local context, the study's population and outcomes should be compared to the target population. The study's limitations, such as short duration and the health status of participants, might affect its relevance to clinical practice. Further research, particularly regarding long-term effects, is needed before widespread application.

Study and citation: Effect of wheat bran derived prebiotic supplementation on gastrointestinal transit, gut microbiota, and metabolic health; A randomized controlled trial in healthy adults with a slow gut transit (Muller et al., 2020).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes ☒ No Can't tell
	
	Double-blind parallel trial.
	Population: 48 normoglycemic healthy Caucasian men and women, 20-55 years. BMI: 20-30 kg/m2. From Netherlands.
	The trial aimed to investigate the effect of wheat bran derived prebiotic supplementation (AXOS) on gastrointestinal transit, gut microbiota, and metabolic health in healthy adults with a slow gut transit.
	Pirmary outcome: AXOS) could influence the whole-gut-tranist-time (WGTT) in adults with slow GI transit but without constipation.
	Secondary outcome for this study were gut microbiota composition and gut hormones among others

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒ No Can't tell The study design specifies that this was a randomized placebo-controlled trial. Block randomization was used by an independent researcher to allocate participants to the AXOS or placebo group. 24 received AXOS, and 24 received placebo. The controlled randomized design helps in minimizing bias and establishing a causal relationship between AXOS and the observed outcomes.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒ No Can `tell Provided in a flow-chart Figure 2 in supplementary. Lost to follow up = 0

Section B: Was the study methodologically sound?

4.	• Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ The study was double-blinded. Both AXOS and maltodextrin (placebo) were provided as powders prepackaged in identical opaque sachets to be dissolved in water, ensuring the blinding. Particpants and investigators were blinded to the intervention. Does not explicity mention if the people assisseing the outcomes were blinded to the intervention.
5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes No Can't tell ☒ This was cleary set out in a table 1 in the result section. There were no differences in BMI, body composition, dietary intake, physical activity and quality of life between groups before or after the intervention.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ Both groups received powders to ingest with meals over 12 weeks, with AXOS for the experimental group and maltodextrin as a placebo for the control group.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?*	Yes No Can't tell ☒ Power calculation was undertaken. To measure clinically relevant differences in WGTT of 30%, a power of 80% (β) and assuming an alpha of 0.05 (α), 22 participants per group were required." This statement indicates that the researchers performed a power calculation to determine the necessary sample size to detect a clinically relevant difference in WGTT (Whole Gut Transit Time) with 80% power and a significance level of 0.05. They estimated a 20% dropout rate, which led them to recruit a total of 48 participants.

• *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	The treatment effect of AXOS over 12 weeks is most pronounced in its impact on the gut microbiota, specifically increasing Bifidobacterium and reducing microbial diversity, while softening stool consistency. However, its effects on metabolic health and GI transit, in this healthy study population, were limited.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes ☒ No Can't tell CI were reported in supplementary Table 5.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes ☒ No Can't tell Increased Bifidobacterium: The AXOS intervention resulted in a significant increase in fecal Bifidobacterium, which has been shown to have potential health benefits. Softer Stool Consistency: A change in the Bristol Stool Score (BSS) indicates a softer stool consistency, which might be beneficial for those with constipation or hard stools. Microbiota Composition: The intervention impacted the overall gut microbiota composition, which might have potential implications in understanding gastrointestinal health. The study does not give explicit information about the financial costs associated with the AXOS intervention, nor does it detail any indirect costs (e.g., time, resources, potential side effects). Potential Harms: Decreased Microbial Diversity: The intervention led to a decrease in microbial diversity. While high microbial diversity is generally seen as a sign of a healthy gut, the exact implications of this decrease in the context of AXOS intake are not completely clear. No Impact on Some Parameters: Several parameters like whole-gut transit time (WGTT), metabolic outcomes like insulin, glucose concentrations, and others were not affected by the intervention. Depending on the objectives of the treatment, this could be seen as a limitation or neutral effect. GLP-1 Reduction: The early postprandial GLP-1 was reduced after AXOS intervention. The implications of this in terms of long-term health would need to be explored.)

Section D: Will the results help locally?	
10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study shows AXOS softens stool consistency, which may be beneficial for some but might not be a primary concern for all. The study didn't show significant changes in whole-gut transit time. If the aim is to primarily address this aspect, then the AXOS intervention might not provide the desired outcome. If the population has a diverse range of gastrointestinal behaviors or includes individuals with known metabolic disorders, their responses to AXOS might be different from the study participants The study population consisted of healthy Caucasian men and women from Maastricht, the Netherlands, aged 20–55 with specific inclusion criteria. The generalizability would depend on how closely the local population matches these criteria. Limiations: A potential limitation is the inclusion of only healthy participants. This might have left limited scope for observing improvements in metabolic health parameters, especially when compared to individuals with metabolic conditions like obesity or type 2 diabetes Some bacteria that were notably different in responders vs. non-responders are not well-studied, limiting the ability to understand or hypothesize their potential role or mechanism of action in the observed outcomes. Despite an increase in Bifidobacterium, there was no corresponding change in short-chain fatty acid levels or many markers of metabolic health, suggesting a disconnect or additional interacting factors that are not understood or measured in this study. There was notable variability in responses to AXOS intake, which might hint towards a role of individual factors (e.g., baseline gut microbiota composition) in determining the impact of dietary interventions.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to*	Yes No Can't tell ☒ To determine whether the AXOS intervention provides greater value than existing interventions, one would need data comparing AXOS to those interventions. This study compare AXOS to a placebo, which provides information on the efficacy of AXOS but doesn't directly compare it to other possible interventions.

be able to re-invest in the new intervention?	

APPRAISAL SUMMARY: *Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?*

Key Findings:
AXOS led to a significant rise in fecal Bifidobacterium, a decrease in microbial diversity, and softer stool consistency, compared to the placebo group.
AXOS intervention showed a significant reduction in GLP-1.

Overall: The study seems well-constructed with clear objectives and rigorous methodology.
No significant change in whole-gut transit (WGTT) after AXOS intervention which were the primary outcome for this study. Secondary outcome for this study were gut microbiota composition and gut hormones.
In summary, the study, while offering valuable insights into the effects of AXOS on gut microbiota and gut hormones, underscores the complexity and perhaps individual-specific nature of these interactions. Future studies might look into a more heterogeneous participant pool or focus on individuals with certain metabolic or GI conditions to explore if AXOS or similar interventions might offer more pronounced benefits in these groups. Furthermore, mechanistic studies exploring the pathways through which AXOS impacts gut microbiota and satiety regulation might also offer further insights into the phenomena observed here.
Limitations:
Only healthy participants were included, potentially limiting the observable improvements in metabolic health parameters, especially when compared to metabolically compromised subjects, such as those with obesity or type 2 diabetes.
Strengths:
The study employed a controlled randomized design and provided a comprehensive phenotypical characterization of participants, considering both subjective and quantitative aspects of GI and metabolic profiles.

Study and citation: The Effect of White Rice and White Bread as Staple Foods on Gut microbiota and Host Metabolism (Mano et al., 2018).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes ☒ No Can't tell
	
	RCT crossover trial.
	Population: 7 healthy volunteers, student and research staff at Kyoto Univeristy, Japan. 31-42 years old. BMI: 18.6-23.1 kg/m2
	The study aimed to determine the effect of two staple foods as white rice and white bread on gut microbiota and host metabolism.
	Primary outcome for this study was changes in the abundance of fecal Bifidobacterium genus, and secondary outcome were fasting GLP-1 levels.
	The study population consists of healthy volunteers from the research department, with specific inclusion and exclusion criteria set.
	The interventions were clearly defined: consumption of either white bread or white rice along with specific supplied side dishes.
	The study is a crossover trial, meaning each subject tried both interventions, making it a self-comparison. They had a bread period and a rice period, each lasting for a week. The outcomes were clearly outlined. Secondary outcomes included host metabolism parameters such as plasma glucose, serum insulin, serum FFA, serum triglyceride, total GLP-1, total GIP, and breath hydrogen.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒ No Can't tell Randomized, crossover trial. The participants were randomized in a 1:1 fashion to one of two intervention sequences: either they would first go through a bread period and then a rice period or vice versa. The method is not explicitly mentioned.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒ No Can `tell Ten healthy volunteers initially participated in the study. However, three subjects had a fever or diarrhea during the test period and were thus excluded from the analysis. The remaining seven healthy subjects were analyzed. So, yes, all participants who entered the study were accounted for at its conclusion.

x

Section B: Was the study methodologically sound?

4. • Were the participants 'blind' to intervention they were given? • Were the investigators 'blind' to the intervention they were giving to participants? • Were the people assessing/analysing outcome/s 'blinded'?	Yes No Can't tell ☒ The participants would not have been blind to the intervention. The study was designed as a crossover trial where participants consumed either bread or rice during specific periods. Given that they had to eat these foods and were even tasked with recording the amount of bread or rice they consumed, they would definitely be aware of which intervention they were undergoing. The methodology does not explicitly mention if the investigators were blind to the interventions. However, given the nature of the study (where subjects had to eat specific foods and record the amount), it seems unlikely that the investigators would be blinded. It would be practically challenging for investigators to remain unaware when guiding the subjects on their diets. The methodology does not explicitly state if the people assessing or analyzing the outcomes were blinded.
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes No Can't tell ☒ Baseline characteristics were clearly set out in a table 1 in the result section. As it's a crossover trial, each participant receives both interventions (bread period and rice period) at different times, separated by a washout period. Thus, the "groups" refer to the same set of participants but at different time points
6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ During test periods, subjects consumed nothing other than the assigned staple food and supplied side dishes. There were also specific guidelines for the run-in and washout periods.

Section C: What are the results?

7. Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?*	Yes No Can't tell ☒ Power calculation was undertaken. The sample size calculation was based on a standardized effect size of 2.5 (breath hydrogen) estimated from a previous study . A sample size of five was needed to provide 80% power to detect this difference at a two-tailed

• *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	significance level of 0.05.Statistical Tests: The statistical tests used include the paired t-test, as mentioned in the "Statistical Analysis" section. They used JMP version 13 for their statistical analyses. The outcomes measured include glucose, insulin, GIP, GLP-1, TG, FFA, various SCFAs, breath H2 and different levels of bacteria. These outcomes were specified in tables and text description. For binary outcomes: The results are expressed as means ± standard deviation. The results were reported for each outcome in each study group (bread versus rice period) and displayed in tables. As the subjects were analyzed for both bread and rice periods (a crossover design), there wasn't a differential dropout between these two interventions. The study does not mention source of bias or measures to control them. P-values were reported for several outcomes.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes No Can't tell ☒ CI is provided in a table in table 2,3, and 4 in the result section.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ Bread consumption led to a significant increase in the abundance of Actinobacteria, particularly the Bifidobacterium genus and Bifidobacterium longum species, compared to rice consumption. This is noteworthy as Bifidobacterium is often associated with positive gut health. The plasma GLP-1 level was significantly higher after the bread period compared to the rice period. No harms reported

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ The study was conducted in Japan, on volunteers from Kyoto University. The study participants are healthy subjects, and their age ranges from 31-42. If considering applicability elsewhere, cultural, dietary, and genetic factors should be considered. It's important to see if your population's diet and lifestyle are comparable. The study only involved ten participants initially, and three were excluded from the analysis. Small sample sizes can limit the generalizability of results. It seems that the study is based on a short-term intervention. The long-term implications of consuming bread versus rice are not covered.

11.	Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes	No Can't tell ☒ Introducing a dietary intervention like switching from rice to bread (or vice versa) might take time in terms of educating the population on the benefits, potential risks, and the reasons for the switch. We need to consider what current dietary, or health interventions are in place. Are any of them less effective or less important than the potential benefits gained from changing between bread and rice consumption.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key findings:
Substituting white rice with white bread for seven days led to a significant increase in fecal Bifidobacterium, fasting plasma GLP-1, and breath hydrogen levels.

The study provides valuable insights into the dietary choices of Japanese people and how they might influence gut health and associated metabolic markers. While it demonstrates the potential benefits of choosing bread over rice, making recommendations would require considering individual dietary habits, overall health goals, and cultural preferences. It might be worth considering these findings when making dietary recommendations or guidelines, especially for populations that traditionally consume rice as a staple food. However, direct implementation without considering broader individual or cultural contexts may be premature.
The study seems to have a clear design, and they've taken measures to ensure consistency during the test periods. The absence of blinding might introduce some biases. The trial's generalizability will depend on the specific characteristics of the local population being considered.

Study and citation: An Almond-Based Low- Carbohydrate Diet Improved Depression and Glycometabolism in Patients with Type 2 Diabetes through Modulating Gut Microbiota and GLP-1; A Randomized Controlled Trial (Ren et al., 2020).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	The study was designed as an RCT to assess outcomes of an almond-based low carbohydrate diet in patients with diabetes type 2.
	Population: 45 participants over 18 years old with type 2 diabetes from China.
	The study aims to assess the effect of an almond-based Low Carbohydrate Diet (LCD) on depression in patients with Type 2 Diabetes Mellitus (T2DM). Secondary outcome were gut microbiota composition and fasting GLP-1 concentration.
	The intervention was clearly specified as an almond-based low carbohydrate diet, with 56/g/day of almonds replacing 150/g of staple food rich in carbohydrates. Control group adopted a low-fat diet. Multiple outcomes were measured, including GLP-1 concentration, and gut microbiota profiles.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ The study states that the participants were randomly allocated to either the intervention or control group using a table of computer-generated random numbers. The study design suggest that randomization was likely sufficient to eliminate systematic bias at the point of enrollment. The study specifies that the random numbers were concealed by someone not responsible for the study, indicating allocation concealment.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ Flow chart figure 1 in the result section. 50 participants with diabetes type 2 were initially recruited. 25 allocated to LCD, three withdrew did not like almonds. 25 allocated to LFD, two withdrew. Losses to follow-up and exclusions after randomization are accounted for. It seems like participants were analyzed in the study groups to which they are randomized, consistent with an intention to treat -analysis. The study did not mention being stopped early.

x

Section B: Was the study methodologically sound?

4.	• *Were the participants 'blind' to intervention they were given?* • *Were the investigators 'blind' to the intervention they were giving to participants?* • *Were the people assessing/analysing outcome/s 'blinded'?*	Yes No Can't tell ☒ The random number were concealed by someone who was not responsible for this study, so both researcher and the particpants were blinded prior to assignment. The study does not specifically mention whether the people who were analyzing the outcomes were blinded or not.
5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't ☒ Baseline characteristics was provided in Table 1. There were no statistically significant differences in any of the parameters between the two groups
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ There was a clearly defined protocol. There is a defined inclusion and exclusion criteria, a washout period before the intervention, and d detailed information in the intervention itself. The data was compared at baseline and at the third month for both groups. It appears that the follow up intervals were the same for each study group. The control group and the intervention group (a-LCD) had different dietary interventions. The control group followed a six-point formula based on the diabetes dietary guideline, whereas the a-LCD group consumed 56 g/day of almonds, replacing some of their staple food. Aside from this difference, both groups seemed to have the same treatments and tests. There's no mention of any other differing treatments or tests between the groups.

Section C: What are the results?	
7. Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes No Can't tell ☒ Can't not find information regarding power calculation. Outcomes measured: HbA1c, BMI, weight, gut microbiota, GLP-1. The outcomes are specified. Results are reported for each outcome at the baseline and at a follow-up (third month) for both groups. The dropout rate seems fairly balanced between two groups, so it might not substantially affect the results. Several statistical tests were used, including independent- samples T -test, Pearson, chi-square, Mann-Whitney U. P-values are reported, and the significance level is clearly identified.
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes No Can't tell ☒ CIs was reported in the result section in table 1, 2, and 3, 5, 6 and 7.
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes No Can't tell ☒ The study did not report om any harm of the participants. The LCD diet seems to have a positive impact on the gut microbiome, and GLP-1. Can't find any cost-effectiveness analysis

Section D: Will the results help locally?	
10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?*	Yes No Can't tell ☒ It the local population consist of T2DM patients with and without depression, then it could be applied to the local population. The study finds that the a-LCD diet is beneficial for both glycometabolism and depression in T2DM patients. If these outcomes are crucial for your local population, especially in terms of managing diabetes and mood, then the findings are directly relevant. The study was conducted on T2DM patients both with

• *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	and without depression. If your local population consists mostly of T2DM patients with clinical depression, the results might not fully apply. Due to limited funding, there could be potential improvements in the method of gut microbiota analysis in the study. Other limitation: relatively small sample size. Some participants withdrew from for various reasons, including dislike of almonds, which could be a limitation in recommending such diet.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ The study was conducted over a three-month period, suggesting that improvements can be seen relatively quickly. GLP-1 increased, and gut bacteria Eubacterium Roseburia and Ruminococcus sig. increased. Implementing a LCD would require educating patients about the diet. Cost should be not much adapting to this diet.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?.

Key findings: LCD diet significantly increased the abundance of Roseburia, Ruminococcus, and Eubacterium compared to LFD group. Additionally, by the third month, the level of Firmicutes was significantly lower in the LCD group compared to the LFD group. Bacteroides also decreased significantly in the LCD group compared to baseline levels. As for gut hormones a significant increase in GLP-1 was observed in the LCD group

Primary Outcome: The major focus was on the improvement of glycometabolism, primarily measured using HbA1c levels.
Secondary Outcomes: The research also examined the effects of a-LCD on weight, BMI, depression scores, gut microbiota regulation, and GLP-1 expression.
The study acknowledged its limitations, including the specific T2DM population used (with and without depression).
The diet could however be relevant for T2DM patients.

Study and citation: 2019 Metabolic phenotype and the gut microbiota in response to dietary resistant starch type 2 in normal weight- subjects: a randomized crossover trial (Zhang et al., 2019).

Section A: Is the basic study design valid for a randomised controlled trial?	

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes No Can't tell
	☒
	A randomized crossover study.
	Population: 19 subjects between 18 and 55 years old from China, with normal body weight, BMI: <24 kg/m2. The secondary outcome for this study were gut microbiota composition and the gut hormones GLP-1 and PYY. The dietary intervention involved a high RS2 diet and an energy-matched control starch diet. The high RS2 diet provided 40 grams of RS2 per day.

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes No Can't tell ☒ The subjects were divided randomly into two groups. An independent researcher performed permuted block randomization and assigned the subjects to the starch supplementations. Yes, the allocation sequence was concealed. The order of starch supplementation was blinded for both the investigator and the participants.
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes No Can `tell ☒ The study started with 22 screened subjects, and 3 withdrew before the first intervention, leaving 19 volunteers who completed the study. The study was not stopped early.

Section B: Was the study methodologically sound?	
4. • *Were the participants 'blind' to intervention they were given?* • *Were the investigators 'blind' to the intervention they were giving to participants?* • *Were the people assessing/analysing outcome/s 'blinded'?*	Yes No Can't tell ☒ The participants and investigators were blind to the intervention they recived. The study provides evidence that at least some of the assessors were blinded. Specifically, "MRI scans were performed by experienced radiologist who were blinded to the intervention groups and laboratory finding.

5.	Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?*	Yes No Can't ☒ Could not find any information about baseline characteristics.
6.	Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes No Can't tell ☒ It seems like both groups received the same level of care apart from the experimental intervention. Cleary described protocol.

Section C: What are the results?

7.	Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes No Can't tell ☒ A power calculation were undertaken. The study aimed for a sample size of 22 subjects to accommodate a 20% drop-out rate, ensuring statistical validity for the results. Several outcomes were measured, included GLP-1, PYY, and gut microbiota composition. The generalized estimating equation (GEE) model and the Wilcoxon singed-rank test were mentioned among others. Yes, P-values are reported alongside the results to indicate statistical significance.
8.	Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported*	Yes No Can't tell ☒ CIs were reported in tables in supplementary information.
9.	Do the benefits of the experimental intervention outweigh the harms and costs?	Yes No Can't tell ☒

CONSIDER: • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	RS supplementation seems to offer numerous health benefits without any significant adverse effects. Size of the Intervention or Treatment Effect: The intervention (RS intake at 40g/d for 4 weeks) resulted in significant reductions in intra-abdominal and subcutaneous fat areas, which are notable given the short duration of the study. No adverese effetcts reported. RS reduced abdominal fat without affecting overall body weight, which might be attributed to an increase in the intestinal weight due to the hypertrophic effect of RS on the intestinal wall. RS elevated serum GLP-1 levels, which plays a role in energy metabolism and promotes insulin secretion, potentially aiding glucose metabolism. Certain bacteria genera, which have been linked to obesity and metabolic disorders in previous studies, were influenced by RS intake. The study suggests the beneficial effects of RS might be mediated through changes in the gut microbiota.

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context? *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes No Can't tell ☒ If the local population consists primarily of normal-weight individuals similar to the subjects in the study, the results are directly applicable. However, if the demographic is predominantly overweight, obese, or has a different health profile, the outcomes may vary. Cultural, genetic, and dietary differences can influence gut microbiota and how one metabolizes and responds to RS. If the population has different dietary habits, genetic backgrounds, or lifestyles, the results might not be directly applicable. This is not stated in the study. This study focused on the reduction of abdominal adiposity in normal-weight subjects, changes in gut microbiota, and the elevation of GLP-1 hormone levels. If the local population is concerned about weight management or gut health, the outcomes might be of importance. Limitations: The study was conducted on normal weight individuals, so the findings may not be applicable to overweight, obese, or underweight population. The research was conducted over four weeks, which might not be sufficient to observe long-term effects and understand sustained impacts of RS intake. Nutrient intake data was based on self-reported dietary records which may contain inherent biases or inaccuracies due to participants' recall or honesty. The discussion emphasizes certain microbial changes but doesn't elaborate on potential interactions or synergistic effects among varied gut microbiota, and satiety regulation.

	Information on what was used as a placebo and how effectively the blinding was implemented and maintained throughout the trial is not provided, which is crucial for interpreting results.
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions? *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes No Can't tell ☒ The study offers promising insights into the potential benefits of RS intake in reducing abdominal adiposity and influencing metabolic parameters. However, careful consideration of the population characteristics, relevance of outcomes, study limitations, and individual variability is essential before applying these findings to a different context. Additionally, further research would be beneficial to validate and tailor the findings to diverse populations The study was conducted over a three-month period, suggesting that improvements can be seen relatively quickly.

APPRAISAL SUMMARY:

Record key points from your critical appraisal in this box. What is your conclusion about the paper? Would you use it to change your practice or to recommend changes to care/interventions used by your organisation? Could you judiciously implement this intervention without delay?

Key findings:

Gut microbiota at genus level were observed. 15 bacterial genera were significantly decreased. A significant increase was observed in GLP-1 at 30 minutes in a meal tolerance test after RS intake compared to the control group. RS consumption did not significantly affect the PYY level. Limitations: The nutrient intake data in the study is based on self-reported dietary records, introducing the potential for subjective bias.