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1 Abstract

Obesity and its related comorbidities are a global issue (Stevens et al., 2012). It is thought that obesity is caused by an excess of energy-dense foods in the diet coupled with a lack of energy expenditure through physical activity. Recent evidence suggests that the trillions of microbiota that inhabit the human gastrointestinal tract affect the absorption of key nutrients and influence energy regulation. Further evidence suggests that the composition of the microbiome which is made up of individual microbiota, is different in obese and lean people. The aim of this review was to determine whether dietary interventions that modify the gut microbiome can be used to reduce the prevalence of obesity. A comprehensive search was performed using Tayler Francis Online, Science Direct, PubMed and Wiley Online Library with a further search of reference lists from identified studies. Seven studies were found which met the inclusion criteria. The results of this study suggest that dietary interventions that include prebiotic supplementation can favourably alter the composition of the gut microbiome. This review has identified several lines of evidence for the potential of dietary interventions to reduce the prevalence of obesity and its comorbidities through modification of the gut microbiome.

2 Introduction

The prevalence of obesity has increased rapidly in the last few decades making it a major global issue (Stevens et al., 2012). If current trends continue it is estimated that by 2030 nearly 60% of the world's adult population (3.3 billion people) will be either overweight or obese (Kelly et al., 2008). The number of obese adults will have risen by 65 million in the USA and 11 million in the UK alone (Wang et al., 2011). This makes it a major public health challenge in both the economically developed and developing parts of the world. While overweight and obesity are more common in economically developed countries, the much larger population of developing countries results in a considerably larger absolute number of individuals affected (Kelly et al., 2008).

It is generally believed that obesity is caused by excess an energy intake relative to energy expenditure (Clarke et al., 2012; Aronne, Nelinson, and Lillo, 2009). The etiology of obesity is complex and includes lifestyle choices as well as genetic, physiologic, environmental, psychological and economic factors which are often interrelated (Aronne, Nelinson, and Lillo, 2009). Because humans have adapted to protect against insufficiency, the exposure to an abundance of energy-dense foods can lead to an increase in energy storage (DiBaise et al., 2010). High caloric processed foods have become more affordable and available and the ever increasing portion sizes can all contribute to overeating (Wright and Aronne, 2012). Jebb and Moore (1999) suggest that there is clear evidence that low levels of physical activity are associated with an increased risk of weight gain and obesity. Wright and Aronne (2012) claim that the level of physical activity has declined significantly over the last few decades. However, while there is some evidence that energy intake has increased and physical activity has declined over the last few decades, accurately measuring these changes has proved difficult as sufficient methods of recording physical activity have not always been reliable (Hill, 2006).

The increase in the consumption of high caloric food coupled with the decline in physical activity helps to create what Swinburn and Egger (2004) describe as positive feedback cycles. Swinburn and Egger (2004) propose that the positive energy balance that leads to obesity is actually quite small and that numerous positive feedback cycles act as accelerators that can lead to an increase in weight. For example, an increase in weight might increase the effort of physical activity making exercise harder which could lead to a reduction in activity levels and other changes in the feedback system leading to a further increase in weight (Swinburn and Egger, 2004). According to Ley et al. (2006), one factor that influences these positive feedback cycles is the microbiome of the gut. Recent evidence suggests that the trillions of microbiota that inhabit the human gastrointestinal tract affect the absorption of key nutrients and influence energy regulation. Further evidence suggests that the composition of the microbiome is different in obese and lean people. These findings show that the gut microbiome is an important factor in health

by regulating metabolism and energy storage and may be partly responsible for the development of obesity in predisposed people.

2.1 Types of gut microbiota

The majority of microbiota in the body are found in the digestive system (Backhed, 2005). The interactions between the microbiota and host occur predominantly along the mucosal surfaces, separated from the internal milieu by a single layer of epithelial cells (O'Hara and Shanahan, 2006). The large intestine contains the most microbiota with up to 10^{12} per gram of contents. The estimated number of microbial species found in the gut varies considerably between different studies, but in general it is recognised that the gut contains 500 to 1,000 different species (Xu and Gordon, 2003) with approximately 7000 different strains (Backhed et al., 2005). Qin et al. (2010) sampled 124 European individuals and estimate that allowing for 15% of prevalent genes that may be missing from the catalogue the number of species within the microbiome could be as high as 1150. Qin et al. (2010) state that any one individual can have up to 160 such species in their gut.

The species that make up the microbiome in the gut include bacteria, archaea, viruses, and unicellular eukaryotes (Sekirot et al., 2010). According to microbial DNA sequencing the human gut microbiome is predominantly made up from four bacterial phyla: Gram-negative Bacteroidetes and Proteobacteria and the Gram-positive Actinobacteria and Firmicutes (Macfarlane and Macfarlane, 2011; Tilg and Kaser, 2011). The ratio of the two major phyla, Firmicutes to Bacteroidetes appears to remain relatively stable over time within individuals (The Human Microbiome Project Consortium, 2012; Faith et al., 2013). However, this ratio can vary significantly between individuals (Lozupone et al., 2012). The most common genera of the bacteria phyla are Bifidobacterium, Lactobacillus, Bacteroides, Clostridium, Escherichia, Streptococcus and Ruminococcus (Conlon and Bird, 2014).

2.2 The functional role of the gut microbiota in health and disease

The gut microbiota have coevolved with the human host in mutually beneficial ways to perform numerous functions that affect the host's physiology (DiBaise, Frank, and Mathur, 2012; Delzenne and Cani, 2011). According to Shanahan (2002) one of the gut microbiome's most important roles is within the functioning of the mucosal immune system. Recent evidence suggests there is a beneficial partnership between symbiotic microbiota and the immune system of the host involving certain signals that are exchanged between the two. The microbiota are able to direct immune responses and in turn the immune system is able to influence the composition of the microbiota (Round and Mazmanian, 2009). The main functions of the gut microbiota in the host's immune system is to provide a physical barrier to pathogens by competing for the occupation of attachment sites, the consumption of nutrients and the production of antimicrobial substances (Sekirov et al., 2010). Bifidobacteria is one type of bacteria that has been shown to reduce the levels of endotoxins circulating the body by improving mucosal barrier function thus reducing inflammation, Lactobacillus has also been shown to reduce inflammation in this way (Mortaz et al., 2015; Tien et al., 2006). Other microbial species are thought to initiate specific T-cell responses. For example, Bacteroides fragilis is able to elicit a systemic anti-inflammatory immune response by inducing changes in the cell chemistry of naive CD4 + T-cells into Treg cells (Cénié et al., 2014).

Studies on individuals with immunodeficiencies have also shown gut dysbiosis (unhealthy microbiome composition) to be present. The results of a study by Smekens et al. (2013) discovered that Acinetobacter was more predominant in subjects with hyper IgE syndrome (a rare immunodeficiency disease) whereas in typical healthy individuals the microbiome is dominated by Firmicutes and Proteobacteria. Relatively few studies have been done in this area to date but this field will most likely develop as the understanding of the gut microbiota increases.

The microbiome has also been shown to have an effect on circulatory diseases. Cholesterol synthesis is increased when acetate, which is produced by some microbiota, is absorbed in the body. However, propionate, which is also produced by the microbiota, has been shown to inhibit cholesterol synthesis. For that reason, maintaining the optimal Acetate to Propionate ratio may reduce the risk of cardiovascular disease (Wong et al., 2006).

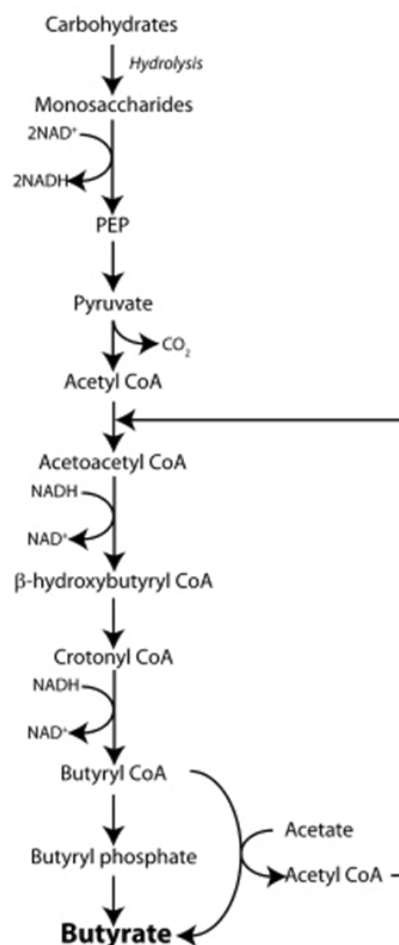
The microbiome of the gut is also responsible for the production of a number of bioactive compounds that influence the host's health. Bacteria such as *Bifidobacterium* can synthesize and supply vitamins such as vitamin K, B12, biotin, folate and thiamine (Nicholson et al., 2012). LeBlanc et al. (2013) state that this process is important as humans do not have the capacity to biosynthesise most vitamins, therefore, this must be done exogenously. Certain gut microbiota produce phytases which are capable of degrading phytic acid which is present in grains, making the minerals such as calcium, magnesium and phosphate more bioavailable (Sandberg and Andlid, 2002). Many of the products of the functions carried out by the microbiota are beneficial to the host. However, some bioactive compounds such as ammonia, phenols and hydrogen sulphide which are produced by the microbiota through the process of protein fermentation can be toxic at high levels (Conlon and Bird, 2014).

It is understood that humans possess approximately 17 digestive enzymes (Turnbaugh, Henrissat, and Gordon, 2010). According to Besten et al. (2013), humans do not possess the enzymes needed to break down the majority of dietary fibres. Therefore, much of the non-digestible carbohydrates pass unaffected through the stomach and small intestine where they are utilised in the anaerobic processes of the microbiota (Mohammed and Guda, 2015).

2.3 Bacterial fermentation and its role in energy production

Fermentation is performed in the large intestine by hydrolysing non-digestible carbohydrates into oligosaccharides and then monosaccharides (Ley et al.,

2005). The monosaccharides are converted into phosphoenolpyruvate (Miller and Wolin, 1996). At this point, nicotinamide adenine dinucleotide (NADH) is formed which acts as an electron carrier (Besten et al., 2013). To remove these excess reducing equivalents, pyruvate is converted to lactate or ethanol lactate (Pokusaeva, Fitzgerald, and van Sinderen, 2011), thus oxidizing the NADH see figure 1. Certain chemoheterotrophic Firmicutes - *Eubacterium hallii* and *Anaerostipes caccae* are capable of utilizing lactate which is produced by many gut bacteria such as *Bifidobacteria* (Flint et al., 2014) and converting it into butyrate (Duncan, Louis, and Flint, 2004) which plays an important role in the maintenance of intestinal homeostasis and overall health status by reducing mucosal inflammation, improving oxidative status, reinforcing the epithelial barrier, reducing obesity and insulin resistance by promoting energy expenditure and stimulating mitochondrial



function (Canani, 2011).

Figure 1. Schematic overview of anaerobic fermentation pathway (Besten et al., 2013)

The fermentation reactions produced in the gut by the microbiota are self-regulating as the reduction-oxidation reaction differential between the substrates and the metabolic end products determine the amount of energy that can be produced (Macfarlane and Macfarlane, 2011). Macfarlane and Macfarlane (2011) suggest that anaerobic fermentation is a relatively inefficient process that provides a low adenosine triphosphate (ATP) yield, therefore a large amount of substrate is necessary. This results in large quantities of metabolic end products being formed such as hydrogen (Krajmalnik-Brown et al., 2012). According to Krajmalnik-Brown et al. (2012), the removal of H^2 from the system influences the symbiosis of the microbes and the host. H^2 is expelled through the breath of the host (Marthinsen and Fleming, 1982) or oxidised by methanogens, acetogens (homo-acetogens), and sulfate reducers in the large intestine (Nava et al., 2011). Gibson, Macfarlane, and Cummings (1993) suggest that it is the processes involving H^2 that are critical for fermentation to continue as the build-up of H^2 in the colon impedes further fermentation. The main short-chain fatty acids (SCFAs) produced from carbohydrate fermentation are acetate, propionate, and butyrate (Wong et al., 2006). It is estimated that the SCFAs produced in this manner provide 10% of the total dietary energy supply (McNeil, 1984).

2.4 Microbiota and its link to obesity

Several studies have shown that alterations in the levels of the gut microbiota may lead to obesity and metabolic syndrome (MetS). It is proposed that these alterations in the gut microbiota cause inflammation that may lead to the development of MetS (Chassaing and Gewirtz, 2013). Research suggests that although lipopolysaccharide (LPS) which is derived from the cell wall of gram-negative bacteria such as Firmicutes is found at relatively low levels in the blood of healthy individuals, in the presence of obesity and other metabolic disorders significant increases in LPS levels can cause metabolic endotoxemia (Cani et al., 2007).

According to the International Diabetes Federation (IDF) (2005) for an individual to be defined as having metabolic syndrome they must have central obesity (defined as waist circumference $\geq 94\text{cm}$ for men and $\geq 80\text{cm}$ for women) plus any two of the following four factors: raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg), dyslipidaemia (increased triglycerides ≥ 1.7 mmol/L) and reduced high-density lipoprotein cholesterol (< 1.03 mmol/L* in males and < 1.29 mmol/L* in females), raised fasting glucose (FPG ≥ 5.6 mmol/L) or specific treatment for any of these conditions.

There is an increasing amount of data showing the association between gut microbiota and metabolic diseases. Metabolic syndrome, diabetes, and obesity are characterized by low-grade inflammation (Tilg and Moschen, 2006). Sartor (2008) suggests that the gut microbiota play a role in inflammatory bowel diseases due to dysbiosis of the microbiota and intestinal inflammation caused by host genetic defects or pathogens that cause alterations in the populations of commensal bacteria and defective host immune responses.

Amar et al. (2011) have demonstrated that high-fat diets can impair epithelial integrity in mice leading to increased gut permeability, and consequently to systemic inflammation via translocation of live commensal intestinal bacteria that are found to be present in large numbers in the blood and adipose tissue where they can induce inflammation by activating immune responses. Other animal studies have shown similar results, Kalischuk, Inglis, and Buret (2009) found that the *Campylobacter*, a non-fermentative bacterium, promotes the translocation of non-invasive bacteria across the intestinal epithelium regardless of its own invasiveness. Gewirtz et al. (2001) found that certain bacteria such as the pathogenic *Salmonella* can translocate flagellin (a structural component of bacterial flagella) across epithelia, thus causing inflammation, when other commensal bacteria such as *Escherichia coli* cannot. This translocation of bacteria has also been confirmed in humans. Reddy et al. (2007) found *E. coli* was able to translocate from the gut to the mesenteric lymph nodes and beyond.

High fat diets are thought to induce insulin resistance in mice by promoting inflammation in adipose tissue by activating a direct immune response to dietary saturated fatty acids (Shi et al., 2006), however, Mohammed et al. (2012) state there is mounting evidence that low-grade inflammation linked to insulin resistance is caused by dysbiosis of the gut microbiome rather than by the response to saturated fatty acids. In their study Mohammed et al. (2012) showed that obese and glucose intolerant mice showed increased concentrations of Immunoglobulin G (IgG) acting against pathogenic bacteria such as *Escherichia coli*. The increased concentrations of IgG correlated with increases in Tumor Necrosis Factor alpha (TNF α) which are thought to down regulate the signalling of insulin receptors (Hotamisligil, 1999). However, it is unclear whether elevated concentrations of IgG are a direct cause or a consequence of insulin resistance.

It is important to note that both these studies relied on results of high fat diets in mice. The diets for these mice consisted of casein, maltodextrin, sucrose, lard, soybean oil and hydrogenated coconut oil which are not the natural diets of mice. According to Warden and Fisler (2008) dietary phytoestrogens from the soybean oil influence blood insulin, leptin lipogenesis and lipolysis and sucrose contributes to insulin resistance and dyslipidemia in mice. These foods are not found in the percentages in the human diet so cannot be used to represent a human diet. Therefore based on these two studies the evidence for a high fat diet in humans causing inflammation must be viewed with caution.

Cani et al. (2007) suggest that bacterial lipopolysaccharide (LPS) may also be responsible for the down regulation as it stimulates the release of several cytokines known to contribute to insulin resistance. From the research it is clear that impaired gut barrier function is an important factor in metabolic disease, however, more research is needed to fully understand the precise mechanisms involved.

2.5 Gut microbiota composition in obese vs lean individuals

Studies have shown that the microbiota that occupy the gut can differ significantly between individuals whether lean or obese. The reason for this diversity is not fully understood although diet, the environment, genetics and early microbial exposure are all thought to be factors (The Human Microbiome Project Consortium, 2012).

Research shows that the composition of the gut microbiota in humans can be a major factor affecting energy balance. De Filippo et al. (2010) compared the microbiome of European children consuming a “modern Western diet” with that of rural African children eating a “high-fiber diet”. There was a significant increase in the number of Bacteroidetes coupled with a decrease in Firmicutes in the African children compared with the European children. From the study the exact composition of macronutrients within each diet was unclear, however, it can be assumed that the modern Western diet can be characterised as being high in fat and in low fibre (Stoll, 1998) consisting of refined sugars, refined vegetable oils, high levels of salt and few vegetables (Cordain et al., 2005). De Filippo et al. (2010) hypothesized that the differences in gut microbiota could be the result of a diet high in fibre although it is possible that environmental factors such as sanitation, hygiene, geography, and climate could also play a role in increasing the metabolic energy extraction from plant polysaccharides.

Studies on the composition of the gut microbiome of obese individuals have shown high levels of Firmicutes and low levels of the Bacteroidetes as opposed to lean individuals where the reverse was the case (Ley et al., 2006; Santacruz et al., 2010). This shift in composition could potentially contribute to increases in body fat from greater energy harvest (Ley et al., 2005; Turnbaugh et al., 2006; Ley et al., 2006). The results reported by Ley et al. (2006) were not supported by Duncan et al. (2008) who found no significant differences in the population of Bacteroidetes during a weight loss intervention. Duncan et al. (2008) showed no change in the percentage of Firmicutes, however, Roseburia and Eubacterium which are bacteria within the Firmicutes phylum did show a reduction. The most striking difference

between the two studies was duration. The study by Ley et al. (2006) lasted for one year whereas the study by Duncan et al. (2008) only lasted eight weeks. Neither study detailed the diets used and the calories within so it is not possible to directly compare the two studies.

Zhang et al. (2009) who demonstrated that the number of Bacteroidetes was greater in obese subjects. In contrast to Ley et al. (2006) Schwartz et al. (2010) claims the ratio of Firmicutes to Bacteroidetes changed in favour of the Bacteroidetes in obese subjects. The difference in the findings of these studies makes the link between the ratio of Firmicutes to Bacteroidetes controversial.

According to a literature review by Delzenne and Cani, (2011) Bifidobacterium and Lactobacilli are lower in obese individuals although they do suggest these findings are often from controversial data although they do not make reference as to why this might be which may indicate a bias on their part. Using rRNA gene sequencing, an analysis of the gut microbiome in twins has shown that obesity is associated with a decrease in the number of Bacteroidetes and an altered expression of genes that lead to an increased energy uptake and adipogenesis (Turnbaugh and Gordon, 2009). Other research suggests that the shifts in microbial populations are a result of calorie dense obesogenic diets (Lin et al., 2012). It is therefore unclear as to whether dysbiosis of the gut causes obesity or whether obesity drives the change in the diversity of the microbiome. As a consequence, if it is obesity that drives the changes in the microbiome, a diet designed to modify the microbiome may have little to know effect on obesity. The composition of the microbiome is both diverse and adaptable and it is clear that dietary factors can significantly affect the overall population and structure of the community within microbiome (Tang and Hazen, 2014).

2.6 Effects of diet on the composition of the gut microbiome

Diet has a major influence on the composition and activity of the gut microbiome. The process of fermentation of carbohydrates and its effect has been outlined above. The effect of dietary fats and protein on the microbiome

is less understood. A typical Western type diet provides the microbiota of the large intestine with approximately 50g of fermentable material, approximately 5–15 g of this is protein and 5–10 g is fat the rest is made up from indigestible carbohydrates (Conlon and Bird, 2014).

Dietary protein is a major source of nitrogen needed for microbial growth and is essential to the microbiota's ability to ferment carbohydrates and thereby produce products such as SCFAs. Studies have shown that a moderate to high protein diet can contribute to weight loss in overweight individuals by improving insulin resistance (Wycherley et al., 2010) which can be caused by changes in the composition of the microbiome (Caricilli and Saad, 2013).

The process of protein fermentation by the microbiota produces a large amount of different gases, metabolites and putrefactive fermentation products (Silvester and Cummings, 1995). These include ammonia and hydrogen sulphide (which have been shown to be cytotoxins), genotoxins and carcinogens (Hughes, Magee, and Bingham, 2000). However, Brinkworth et al. (2009) found that higher protein intake does not necessarily result in higher levels of protein fermentation products. Brinkworth et al. (2009) suggests that previous research has shown that increased protein intake increases the amount of protein reaching the colon from the small intestine increasing the levels of protein fermentation products. However, Brinkworth et al. (2009) did not observe this in their study hypothesising that the restriction of energy in the diet may have improved the efficiency of the small intestine by absorbing more of the available protein, thereby reducing the amount of protein that reached the colon. Although ammonia is known to be toxic at high levels, it is used as a source of nitrogen by the microbiota and is mostly absorbed in the gut or excreted via stool or in urine and therefore is unlikely to reach significantly high levels.

Graf et al. (2015) suggest that fat quantity as well as fat quality and type of fat affect the composition of the gut microbiota via their impact on the secretion of bile acid as well as the bile acid composition. Laugerette et al. (2011) state that few studies investigating the effect of dietary fat take into account the different types of fat available in Western diets. Laugerette et al.

(2011) studied the effects of different fats and oils typically found in the Western diet and showed that each had different effects on inflammation in the body. Palm oil was shown to increase concentrations of cytokines in plasma. Conversely milk fat resulted in a lower inflammatory profile than the palm oil, even though they are similarly rich in saturated fatty acids. Two possible hypotheses were given: depending on their nature, long-chain saturated fatty acids, n-6 and n-3 fatty acids have different mechanisms that are responsible for their metabolism, leading to different inflammatory processes or the different fatty acids can bring about different metabolic changes that can lead to changes in lipid and LPS absorption. Laugerette et al. (2011) stress the importance of these findings as vegetable oils, including palm oil, are now increasingly being cited as healthier alternatives to animal fats such as milk fat. A study by Austin et al. (2009) found that individuals with irritable bowel syndrome (IBS) had a reduction of symptoms on a high fat (51% of calories) high protein (45% of calories) diet in which carbohydrates were limited to 20 grams per day. Paoli et al. (2015) studied the effects of a ketogenic diet on food intake. The authors found that this type of diet led to adaptations in the microbiota to the absence of carbohydrate where the body shifted to the use of ketones for energy. Paoli et al. (2015) also hypothesised that ketosis could aid weight and food control due the butyrate production by gut bacteria and the circulation of high numbers of ketones.

Butyrate supplementation using sodium butyrate has been shown to prevent the development of diet-induced obesity and insulin resistance in mice. It was also shown to reduce obesity and insulin resistance in obese mice without reducing food intake or fat absorption (Gao et al., 2009). Gao et al. (2009) propose that the anti-obesity effect was due to an increase in energy expenditure and the resulting reduction in adiposity led to the reduction of insulin resistance. Lin et al. (2012) found that butyrate reduced diet-induced obesity without causing a reduction in food intake in mice. The obesity reducing effect of butyrate producing gut microbiota such as *Lactobacilli* may also be due to the their ability to bind to the surface of colonic epithelial cells

thus providing a protective barrier to pathogens and reducing inflammation (Ouweland et al., 1999).

Although the understanding of energy metabolism and the inflammatory reducing effects of the gut microbiota is increasing, the exact mechanisms and effects of their metabolic end products are less clear. Further research is required on the interactions between dietary fat and the composition and function of the microbiota in relation to energy metabolism and obesity.

2.7 Therapeutic implications of gut microbiome modulation using pro- and prebiotics

There is growing interest in the role of the gut microbiota in the incidence of obesity and its comorbidities. Nutritional interventions focused on pro- and prebiotics are potential ways of modulating the gut microbiota to improve gut health and reduce obesity and systemic inflammation (Delzenne and Cani, 2011). Delzenne and Cani (2011) do, however, caution that much of the evidence is based on controversial data, which casts doubt on their validity.

It is difficult to summarise much of the evidence relating to the use of probiotics as the methods used and the results differ greatly. Much of this is due to the specific targeting of certain bacterial genera (Ceapa et al., 2013). The transient effect of probiotics is understood not to have a particularly effective role in making long-term changes to the global diversity of the microbiome but can be beneficial in modulating epithelial receptors to influence the immune system (Savard et al., 2011).

Much of the research involving prebiotics focuses on the use of inulin-type fructans (ITF) and the galacto-oligosaccharides (GOS) (Ceapa et al., 2013). Many of these studies support the hypothesis that the introduction of these prebiotics in the diet can modify the composition of the gut microbiome particularly the number of *Bifidobacterium* (Roberfroid et al., 2010) shown to improve immune function. There is a growing amount of evidence that shows that dietary interventions aimed at modifying the gut microbiome can be used to improve health markers associated with obesity. However, much of the

evidence is based on correlations and has been unable to establish a cause–effect relationship (van Hylckama Vlieg et al., 2011).

2.8 Issues relating to murine models for human gut microbiota research

The growing field of research into the function of gut microbiota and its association with obesity has relied heavily on studies using animals, most commonly murine (mice and rats). While it is widely recognised that animal models have contributed greatly to the understanding of the effects of diet on obesity (Speakman et al., 2008; Kanasaki and Koya, 2011; Vickers, Jackson, and Cheetham, 2011), the symbiotic relationship between the gut microbiota and host (Malys, Campbell, and Malys, 2015) and the effect of the microbiota on metabolism and metabolic disease (Ley et al., 2006), caution is needed when translating the results to humans as although there are similarities in the two biological systems, there are also marked differences that can make the experiments non-transferable from murine to humans (Nguyen et al., 2015). Physiological differences include the lack of a gall bladder in rats (Vickers, Jackson, and Cheetham, 2011) and differences in the structure of the colon (Nguyen et al., 2015). Kanasaki and Koya, (2011) draw attention to the issue that although many current animal models of obesity now exist, models for some obesity-associated human diseases have yet to be established. Many animal studies are poorly designed, conducted and analysed and reviews of animal research are often methodologically inadequate (Bracken, 2009).

2.9 Aim of this study

In recent years, there has been an increasing amount of literature suggesting that diet can influence the composition and diversity of the gut microbiome and that negative shifts in the composition of the microbiome can lead to weight gain and obesity. It has been proposed that the gut microbiome plays a causal role in obesity (Harley and Karp, 2012). According to Harley and Karp (2012) there are a number of reasonable, biological mechanisms that may overlap including microbiota with an increased capacity for energy

harvest, direct modification of host energy uptake, utilization and/or storage of microbial metabolites and actions of the microbiota on the host immune system.

A large number of reviews exist in the field of the gut microbiota and its links to obesity, however, these are of a descriptive nature. A preliminary search performed as part of this review revealed a lack of systematic reviews on the subject. This may be due to the fact that this is a fairly new field of research and that until recently there has not been the body of research to warrant a review of this type. Now, thanks to advances in DNA sequencing technologies, a comprehensive examination of microbial communities sampled from natural environments, without the need for cultivation, is possible. This has led to an increase in the number of studies investigating the associations between the microbiota and the host.

Systematic reviews differ from descriptive, narrative reviews in that they are intended to remove much of the bias that can be found in descriptive reviews (Higgins and Altman, 2008). A comprehensive systematic approach aids to synthesise evidence from a large body of research thus making it easier for practitioners and health care professionals to make informed decisions based on quality evidence (Levin, 2001). By scrutinising the methodology and analysing the results from many studies, a consensus can be reached as to the best type of intervention suited to a given population with the potential of saving both lives and resources (Freeman, Williams and Dellavalle, 2006). According to Hemingway and Brereton (2009), those who undertake a systematic review should aspire to apply the same level of quality of methodology and analysis to the review of research as should be applied to the studies included. In this study, the aim was to evaluate whether dietary interventions designed to modify the gut microbiome can be used to reduce the prevalence of obesity.

3 Method

The method for this review is consistent with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement (Moher, 2009) where applicable.

3.1 Search Strategy

A preliminary search was performed to will select appropriate keywords and phrases to be used. A comprehensive search was then performed in Tayler Francis Online, Science Direct, PubMed and Wiley Online Library with a further search of reference lists from identified studies. The Boolean Operator (AND) and asterisk were used to refine the searches. The searches were limited to title and abstract and used the search terms; Dietary Intervention Microbiota AND Microbiome Gut Obesity*. Due to the number of studies identified in the preliminary search only studies published in English from 2010 to 2016 were considered. This was done to ensure the relevance of the studies in relation to the technical advances in the genome sciences (Turnbaugh et al., 2007). Filters for study type were used where possible.

3.2 Inclusion/exclusion criteria

As this review was focused on dietary changes to modify the gut microbiota in humans for the reasons stated above all animal trials were excluded. The inclusion criteria were trials based on prebiotic dietary interventions using adults (aged ≥ 18 years) due to maturation issues and the interpretation of BMI results (CDC, 2011) categorised as being overweight or obese (BMI ≥ 25 and ≥ 30 respectively).

The focus of the study was primarily weight loss and improving biomarkers in the general overweight/obese population, so studies on pregnant women, people with eating disorders, and treatment for a specific medical disorder other than those associated with obesity e.g. metabolic syndrome were not included. Studies that included surgery or medication were excluded. Outcome measures included were BMI, MetS biomarkers and compositional changes in the composition of the gut microbiota.

3.3 Data collection

The selected studies went through a three-stage selection process in order to satisfy the inclusion criteria. Studies were included or rejected on the relevance of the title, abstract or after a review of the full text. The data

extracted from the studies were; population characteristics where stated (age, sex, BMI), sample size, diet or prebiotic used, dosage, placebo or control used, intervention duration including follow-up and outcome measures (BMI, weight loss, Biomarkers, microbiota composition).

3.4 Study validity

A hierarchy based on study design (Petrisor and Bhandari, 2007) was used to establish the validity of the studies. All studies were evaluated using the EPHP quality assessment tool for quantitative studies (Thomas et al., 2004) which consists of six criteria: selection bias, study design, confounders, blinding, data collection methods and withdrawals and drop-outs. Each of the criteria is scored strong, moderate or weak and totalled to give an overall rating. The EPHP tool is capable of being used in both randomised and non-randomised studies and was considered to be appropriate to use in systematic reviews by Deeks et al. (2003). A CASP checklist (Critical appraisal skills Programme, 2013) was used to determine the quality and appropriateness of the randomised studies included. Assessment criteria include study design, blinding of participants, methods of data collection and numbers of drop outs. The checklist included eleven questions that could be answered 'yes', 'can't tell' or 'no'. Each 'yes' answer was scored one point each 'can't tell' or 'no' was scored zero points. The scores were calculated for each study. According to the criteria, the studies were then rated in order from 'strong' to 'weak'. Any conflicts of interest were considered and mentioned in the results and discussion section. The CASP checklists have had several updates and are considered to be an appropriate tool for assessing studies (Critical appraisal skills Programme, 2013). The methodological index for non-randomized studies (MINORS) (Slim et al., 2003) was used to determine the quality and appropriateness of the non-randomised self-controlled studies. MINORS contains 12 items scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate). The ideal score is 16 for non-comparative studies and 24 for comparative studies. MINORS is readily usable, reliability and is described as a valid tool for assessing the quality of non-randomized studies (Slim et al., 2003).

4 Results

4.1 Search results

A total of 1420 studies were identified through the database and reference list searches. Of these 1377 were excluded after reading the titles and abstracts. 15 were excluded as they were not available in full text leaving 22 articles to be reviewed in full text. Of the 22 full-text articles, 15 did not meet the criteria for inclusion. A total of seven studies met enough criteria to be

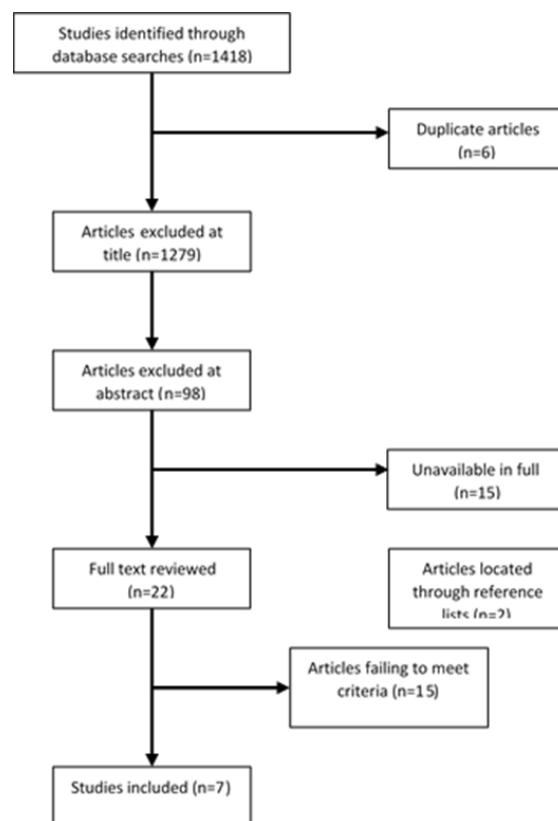


Figure 2. Flowchart of selection studies

included. The selection process is outlined in Figure 2.

4.2 Characteristics of Included Studies

The results of the study selection are summarised in Table 1. Of the seven trials the studies by Lambert et al. (2016, Lappi et al. (2013) and Bañuls et al. (2015) were randomised. Lambert et al. (2016), Dewulf et al. (2012) and

Bañuls et al. (2015) were double blinded, and the studies by Xiao et al. (2013, Remely et al. (2015) Simões et al. (2013) were self-controlled. Sample sizes range from 16 to 123 participants. Study length ranged from 12 weeks to 52 weeks. There were 20 dropouts throughout all the studies. Two of the studies used a diet only intervention (Remely et al., 2015; Simões et al., 2013), one study used diet and a prebiotic supplement ((Xiao et al., 2013) and four studies used a prebiotic diet only intervention (Lambert et al., 2016; Dewulf et al., 2012; Lappi et al., 2013; Bañuls et al., 2015). Both the diet only studies (Remely et al., 2015; Simões et al., 2013) included either exercise and lifestyle counselling or physical activity/exercise sessions. The study by Bañuls et al. (2015) used a high fibre supplement and L-carnitine supplementation and did not measure gut microbiota.

4.3 Quality assessment

Using the EPHPP assessment tool the studies by Dewulf et al. (2012) and Lappi et al. (2013) were scored as strong, four were scored as moderate (Xiao et al., 2013; Lambert et al., 2016; Remely et al., 2015; Bañuls et al., 2015) and Simões et al. (2013) scored weak. All studies scored moderate on blinding and data collection methods. Four of the studies scored strong on study design (Lambert et al., 2016; Dewulf et al., 2012; Lappi et al., 2013; Bañuls et al., 2015) with (Xiao et al., 2013; Remely et al., 2015; Simões et al., 2013) scoring moderate. The weakest criteria was confounders where five studies scored weak (Xiao et al., 2013; Lambert et al., 2016; Remely et al., 2015; Simões et al., 2013) The study by (Dewulf et al., 2012) scored moderate and (Lappi et al., 2013) scored strong. The study by Simões et al. (2013) scored weak in 'withdrawals and drop-outs'. All others scored strong. Of the studies assessed with the MINORS tool two scored 14 points (Xiao et al., 2013; Simões et al., 2013) and one scored 10 points (Remely et al., 2015). Of those studies assessed with the CASP checklist one scored nine points (Lambert et al., 2016), two scored eight (Lappi et al., 2013; Bañuls et al., 2015) and one scored six (Dewulf et al., 2012). The strongest randomised study was (Lambert et al., 2016) and the strongest self-controlled study was (Xiao et al., 2013).

Table 1. Characteristics of included studies

Title	Authors	Year	Population	Type of study	Duration	Methods	Drop out / sample size	Limitations	Main findings	Validity score
A gut microbiota shaped dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome	Xiao et al.	2013	25-55 years 123 total (69 female) (54 male)	Self-controlled clinical trial	23 weeks intervention (9 weeks, Phase I) maintenance period (14 weeks, Phase II).	Formula 1: Corned Gruel Formula 2: powder preparation for infusion Formula 3: Soluble prebiotics	0	89 No control group	- Average weight loss 5.79 ± 4.54 kg (6.5% $\pm$ 4.54%) - BMI significantly reduced from 31.5 ± 0.0 to 29.3 kg m^{-2} ($p < 0.01$). - Participants with Metabolic Syndrome lost from 60.67% to 29.41%. - Reduction in systemic inflammation - Increased insulin sensitivity - Increased insulin sensitivity - Gut permeability decreased - Gut microbiota changed significantly - The relative abundance of Acidobacteria increased significantly from 0.70% to 0.97% - Proteobacteria significantly decreased from 5.29% to 2.25% ($p < 0.01$) - Proteobacteria (gut barrier protecting) significantly increased from 0.51% to 0.53% - Proteobacteria (gut barrier protecting) significantly increased from 0.51% to 0.53% - Enterobacteriaceae (reduced) significantly from 2.84% to 0.97% ($p < 0.01$) and Desulfovibrio (reduced) significantly (ferroplasma producing pathogens) - No significant changes in the abundance of Bacteroides and Firmicutes	- Minos 14 - EPHAPP - Moderate
Consuming yellow pea fiber reduces voluntary energy intake and body fat in obese weight-loss adults in a 12-week randomized controlled trial	Lambert et al.	2014	44±15 years 50 total (41 female) (9 male)	Randomized double blind, placebo controlled, parallel group study	12 weeks	15 g/d yellow pea fibre supplement or isocaloric placebo (n = 30/group)	6	44 Subjects were on self-selected diets	- Pea fibre group lost weight (-0.87 ± 0.37 kg), primarily as fat mass (-0.74 ± 0.28 kg). Placebo group gained weight (0.4 ± 0.38 kg, $p = 0.02$) and fat mass (0.42 ± 0.38 kg, $p = 0.04$). - BMI 33.1 ± 1.3 to 32.8 ± 1.3 - BMI was reduced slightly in the PF group but not in the PL group ($p = 0.025$), but there was no significant change in waist circumference in either group. - No significant change in plasma lipid levels or most inflammatory markers - Fasting adiponectin decreased 7% - Fasting adiponectin and insulin increased ($p < 0.001$ for both outcomes) - No significant effects of pea fibre supplementation on the microbial groups analysed	- CASP 9 - EPHAPP - Moderate
Gut microbiota composition correlates with changes in body fat content due to weight loss	Remely et al.	2015	43±13.65 years 33 obese no vitals	Self-controlled from a prospective study	17 weeks	Dietary intervention consisting of 3 phases according to different diet plans (daily movement and exercise sessions)	0	33 No control group	- BMI decreased from the first (46.5±4.11 kg/m²) to the second time point (42.2±0.33 kg/m²) and remained nearly the same to the third time point (42.3±0.41 kg/m²). - Body fat proportion from (46.5±4.11% of body weight) to (44.0±4.35%, 77-73, $p = 0.02$). - No other biomarkers tested - Significant increase in total bacteria abundance ($p < 0.01$) - Ratio of Firmicutes/Bacteroidetes significantly decreased during the dietary intervention period from T1 to T2 ($p < 0.01$) - Lactobacilli significantly increased between the first and the second time point ($p < 0.01$) - Bacteroidetes showed no remarkable changes - Proteobacteria and Bifidobacteria showed no significant alterations.	- Minos 10 - EPHAPP - Moderate

Table 1. Continued

Impact of a very low-energy diet on the fecal microbiota of obese individuals	Smilke et al 2014	16 total 6 males 10 females BMI 30 kg/m ²	Self-controlled clinical trial	52 weeks	VLCD high in protein and low in carbohydrate followed by a personalized diet plan, combined with exercise and lifestyle counseling	0	16	No control group	Height loss average 5 kg over 52 w at the baseline BMI decreased from 34.5 ± 2.6 to 31.4 ± 4.1. At baseline, participants started with a mean BMI of 34.5 ± 2.6. At baseline, the number of Bacteroides spp. increased. Bacteroides had the most dramatic reduction (approx. 2 logs) followed by the Lactobacillus with a reduction of approx. 1 log. After personalized diet plan Bacteroides increased 1.2 logs and Lactobacillus increased 0.6 logs. The change in numbers of the bacterial groups studied followed the dietary intake and not the weight variations during the 12-month intervention.	Wk 52 Wk 52 Wk 52
Is it right into the metabolic concept: lessons from an exploratory, double-blind intervention study with multi-type nutrients in obese women	Devuyst et al 2013	18-65 years BMI >30 kg/m ² 44 women	A double-blind, placebo-controlled	13 weeks	daily supplement of 16 g (8 g twice a day) of TFF (Synge 1, name) / inulin/dextranose 50/50 (n) or placebo (n placebo arm)	6	30	Systematically assigned groups. Not randomized	No significant impact on BMI and waist/hip ratio but tended to decrease fat mass over time. Different values were not significantly different between both groups. No significant change in HbA1c. No significant change in fasting glycemia, insulinemia or adiponectinemia. Significant decrease in post-OGTT glycemia (figure not available). No effect on cholesterol and triglycerides. No effect on C-reactive protein. Significant reduction in metabolic syndrome exact figure not available. Significant increase in particles from 28.9% to 37.2% (P=0.05) and a decrease in Bacteroides from 67.2% to 57.5% (P=0.05). An increase in Bacteroides and a decrease in Bacteroides occurred after 13-week treatment but not after placebo treatment.	CASP 6 ER-HP ER-HP Strong
Impact of Whole-Grain and Fiber-Rich Rye Bread Versus Refined Wheat Bread Does Not Differentiate the Ethical Microbiota Composition in Finnish Adults with Metabolic Syndrome	Lappi et al 2013	51 total 25 males 26 females BMI of 26–39 kg/m ² 40–65 years	randomized, parallel	12 weeks	dietary intervention: high fiber vs low fiber	0	51	Not stated	The weight of the participants remained the same throughout the intervention (35 ± 14 kg). No difference in metabolic syndrome markers. No difference in glucose metabolism. Improvement in inflammation markers. Improvement in high sensitivity C-reactive protein. Microbiota composition did not significantly differ between the groups after the intervention. Within the WMB group, the participant's microbiota showed a trend (P = 0.06) of Bacteroides spp. (after effect) to increase in the members of Clostridium cluster IV (primarily) as well as Clostridia and Akkermansia. Significant reduction of BMI in both groups of patients. Placebo: With metabolic syndrome (28.8 ± 4.4 to 28.2 ± 4.4). Enriched With metabolic syndrome (28.7 ± 2.6 to 28.0 ± 2.2). Without metabolic syndrome (26.5 ± 2.3 to 25.9 ± 2.7). reduction in blood pressure. reduction in cholesterol and triglycerides. reduction in fasting glucose. reduction in C-peptide. improved insulin sensitivity in WMB only. Suggesting improvement in cardiovascular risk factors. No microbiota transfer.	CASP 6 ER-HP ER-HP Strong
The consumption of a bread enriched with dietary fibre and L-carnitine improves glucose homeostasis and insulin sensitivity in patients with metazoic syndrome	Bakula et al 2015	54 total 28 with MS 26 without MS	double-blind, randomized placebo-controlled trial	12 weeks	high fibre + L-carnitine or control	6	48	Not stated		CASP 6 ER-HP ER-HP Weak

4.4 Body composition

Five of the studies showed a decrease in BMI (Xiao et al., 2013; Lambert et al., 2016; Remely et al., 2015; Simões et al., 2013; Bañuls et al., 2015), of those five studies Xiao et al. (2013) and Bañuls et al. (2015) described the decrease as significant. The decrease in BMI ranged from 0.7 – 3.38 kg/m², however, Bañuls et al. (2015) showed a reduction in BMI in both the intervention group and the control group see Table 1 for detailed results.

4.5 Biomarkers

Out of the seven studies five measured biomarkers (Xiao et al., 2013; Lambert et al., 2016; Dewulf et al., 2012; Lappi et al., 2013; Bañuls et al., 2015). Two showed a reduction in metabolic syndrome biomarkers (Xiao et al., 2013; Bañuls et al., 2015) and three showed no significant reduction in markers (Lambert et al., 2016; Dewulf et al., 2012; Lappi et al., 2013). The study by (Xiao et al., 2013) showed a reduction in systemic inflammation, however, the studies by Lambert et al. (2016) and Lappi et al. (2013) found no reduction. Two studies showed improvement in insulin sensitivity (Xiao et al., 2013; Bañuls et al., 2015) and (Xiao et al., 2013) showed a decrease in gut permeability, see Table 1 for detailed results.

4.6 Effects of diet on microbiota

Both studies by Remely et al. (2015) and Simões et al. (2013) found an increased abundance of total bacteria with the result from Remely et al. (2015) being significant ($P < 0.01$). Remely et al. (2015) found a decrease in the number of Firmicutes but no remarkable changes in Bacteroidetes. However, Simões et al. (2013) showed an increase in Bacteroidetes whereas the total number of bacteria decreased. The study by Simões et al. (2013) found a decrease in Bifidobacteria (2 logs) and Lactobacillus (1log) after the very low energy period followed by subsequent increases in both Bifidobacteria (1.2logs) and Lactobacillus (0.6 logs) following the personal

diet plan phase. Conversely, Remely et al. (2015) found a significant increase in Lactobacilli ($P < 0.01$) but no alteration in the numbers of Bifidobacteria.

4.7 Effects of prebiotics on microbiota

The results from Dewulf et al. (2012) showed a significant change in the ratio of Firmicutes to Bacteroidetes with an increase in Firmicutes and a decrease in Bacteroidetes. Conversely Three studies found no significant changes in the ratio of Firmicutes to Bacteroidetes (Xiao et al., 2013); Lambert et al., 2016; Lappi et al., 2013). However Lappi et al. (2013) showed a decrease of Bacteroidetes and an increase in Firmicutes within the refined wheat bread group. Xiao et al. (2013) found a significant increase in the relative abundance of Actinobacteria and Bifidobacterium and a significant decrease in the abundance of Proteobacteria. Dewulf et al. (2012) showed an increase in Bifidobacterium and Lactobacillus see Table 1 for detailed results.

5 Discussion

There is a growing volume of published studies describing a link between the gut microbiota and the occurrence of obesity. According to Delzenne and Cani (2011) the general consensus of the literature is that the gut microbiota directly influence immunity, nutritional status and contribute to health maintenance. What is less understood are the mechanisms involved and the specific microbiota responsible. Nutritional interventions designed to modify the composition of the gut microbiota thus improving body composition and bio markers associated with obesity and MetS are a potential tool to further understand how specific strains of the gut microbiota influence weight gain in individuals. This is the first systematic review to investigate whether dietary interventions can be used to reduce obesity by modifying the gut microbiota.

Due to the differences in the interventions used in the included studies it is not possible to arrive at a definitive conclusion as to whether diets designed to modify the gut microbiome can reduce obesity. This is due mainly to the variances in the diets used, the different prebiotics and high fibre foods, the duration of the studies and the design of the studies.

Overall the results from the studies showed a reduction in weight and BMI. Only the study by Lappi et al. (2013) showed no change in body weight. In this study the daily intake of energy increased by 1040kj (<0.05) in the rye bread group and 1390kj ($p < 0.01$) in the white wheat bread group, however, the authors state that the percentage of energy from the energy yielding nutrients did not change except for PUFA which decreased. The main difference between the groups in Lappi et al. (2013) was the increase in whole grains in the rye bread group and a decrease in whole grain in the white wheat bread group which was shown not to have an effect on the microbiota. All the other studies showed a reduction in weight with five showing a reduction in BMI (Xiao et al., 2013; Lambert et al., 2016; Remely et al., 2015; Simões et al., 2013; Bañuls et al., 2015). The daily amount of calories ranged from 800 kcals to 2052 kcals. The reduction in BMI in Bañuls et al. (2015) was as a result of the calorie restricted four week run-in period independent of treatment. However, due to the differences in calories in the diets used in the studies it is not possible to determine whether the weight loss was due to calorie restriction, the type of diet or the addition of the prebiotics.

The results of this review show that the studies by Remely et al. (2015) and Simões et al. (2013) using diet only interventions found a decrease in the ratio of Firmicutes to Bacteroidetes. This is consistent with the findings of Ley et al. (2006) who found that after a dietary intervention focused on weight loss, the ratio of Firmicutes to Bacteroidetes more closely resembled that of a lean individual. It is interesting to note that Simões et al. (2013) found that following the very low energy diet the total number of bacteria decreased except for the Bacteroides. The authors of this study suggest that this could have been due to the lack of available substrate from the low intake of carbohydrates. It was only when the trial moved to the next stage where the participants were given a personal dietary plan where the calories increased from 800 kcals/day to approximately 1500 kcals/day that the number of total bacteria increased. This change in bacterial composition according to the author was due to the changes in dietary intake and not the subject's weight loss. The composition of the personal plan was not mentioned so it is not

clear whether it was the increase in calories alone or the specific type of calories i.e. calories from carbohydrates that was responsible for the increase in total bacteria. In contrast to this, Remely et al. (2015) used diets of differing types that contained adequate calories, were high in fruits and vegetables and low in fat and animal products. According to the authors of this study the changes in the ratio of Firmicutes to Bacteroidetes correlated with the reduction in weight of the subjects. This is also consistent with Ley et al. (2006) who state that the changes in the microbiota composition correlated with the changes in weight and not with changes in dietary calorie content of the diet.

Of the studies that used diet plus prebiotic supplementation or prebiotic only interventions, Xiao et al. (2013), Lambert et al. (2016) and Lappi et al. (2013) all found no significant changes in the ratio of Firmicutes to Bacteroidetes which does not support the findings of Remely et al. (2015), Simões et al. (2013) or Ley et al. (2006). The studies by Lambert et al. (2016) and Lappi et al. (2013) were shorter in duration than the diet interventions by Remely et al. (2015), Simões et al. (2013) and Ley et al. (2006) which may indicate these changes in composition may take a longer period of time to occur. However this does not explain the results from the study by Xiao et al. (2013). These studies also contradict the findings of Dewulf et al. (2012) who showed an increase in Firmicutes and a decrease in Bacteroidetes which and the findings of Schwartz et al. (2010) who demonstrated that the ratio of Firmicutes to Bacteroidetes in obese individuals favoured Bacteroidetes.

The Firmicutes to Bacteroidetes ratio is understood to be a marker for health as seen in obese versus lean individuals. An imbalance of this ratio has been associated with different diseases (Ley et al., 2006; Santacruz et al., 2010). In obese individuals the ratio is higher in Firmicutes compared to normal-weight individuals (Ley et al., 2005; Turnbaugh et al., 2006; Ley et al., 2006). (Cani et al., 2007) suggest that the increase in Firmicutes leads to an increased capacity for energy harvest from food and causes low-grade inflammation and the decrease in Bacteroidetes are thought to result in increased plasma LPS levels which can also lead to inflammation (Cani et al., 2007). The results of this present study show that although the

mechanism for the change in ratio of Firmicutes to Bacteroidetes was different between the studies by Remely et al. (2015) and Simões et al. (2013) the overall result was the same. These two studies support the theory that obesity is associated with changes in gut microbiota composition that cause gut dysbiosis which can lead to weight gain and obesity. None of the studies using only prebiotic supplementation showed a positive change in the ratio which suggests that this change in ratio may only have occurred as a result of calorie restriction interventions and that supplementing with prebiotics may have little effect on correcting this balance. Although the ratio of Firmicutes to Bacteroidetes may be used as a marker for health as stated previously it is not possible at this time to say with any confidence whether using these microbiota as the target for modification will result in reducing obesity or improving associated conditions. More research is needed to understand whether the change in gut microbiota drives weight gain and obesity or whether weight gain and obesity causes changes in the composition of the microbiota.

The studies by Dewulf et al. (2012) and Simões et al. (2013) showed an increase in Bifidobacterium and Lactobacillus. Conversely Remely et al. (2015) found a significant increase in Lactobacilli but found no alteration in the numbers of Bifidobacteria. These findings suggest that both diet and prebiotic supplementation can increase the numbers of Bifidobacteria and Lactobacilli which have been shown to improve mucosal barrier function (Griffiths et al., 2004), thought to reduce inflammation (Mortaz et al., 2015; Tien et al., 2006) which is linked to obesity (Canani, 2011). Interestingly, of the studies that measured inflammation only Xiao et al. (2013) showed any reduction in systemic inflammation, the studies by Lambert et al. (2016) and Lappi et al. (2013) found no reduction. Three of the studies support the literature that suggests that diet and or prebiotic supplementation may be beneficial in increasing the numbers of Bifidobacteria and Lactobacilli (Roberfroid et al., 2010) thought to reduce inflammation caused by endotoxemia producing bacteria such as Firmicutes. The study by Xiao et al. (2013) was a diet based on whole grains with traditional Chinese medicinal foods and a prebiotic supplement and lasted 23 weeks whereas the study by

Lambert et al. (2016) used a yellow pea fibre supplement and Lappi et al. (2013) used a high fibre bread versus a low fibre bread. Both the studies by Lambert et al. (2016) and Lappi et al. (2013) lasted only 12 weeks. These results show that although the numbers of Bifidobacteria and Lactobacilli can be increased by diet or prebiotic supplementation the anti-inflammatory effects associated with them was only evident after a diet plus prebiotic supplementation intervention of longer duration. More research is needed to fully understand the anti-inflammatory actions of the gut microbiota and to identify the specific bacteria responsible.

In relation to health biomarkers, including those for MetS, the results of the studies were mixed. Only two studies showed significant reductions in metabolic syndrome biomarkers (Xiao et al., 2013; Bañuls et al., 2015). Although the other three studies that measured biomarkers (Lambert et al., 2016; Dewulf et al., 2012; Lappi et al., 2013) showed no significant reduction, Lambert et al. (2016) suggested there were signs that glucose tolerance was maintained or improved in the fibre group and Dewulf et al., 2012 found a significant difference in the post-OGTT glycaemia with an increase in the placebo group and a slight decrease in the intervention group and Bañuls et al. (2015) found a decrease in fasting glucose levels. When looked at together, although only one study found significant improvements relating to blood glucose, four out of the five studies that measured glucose levels found some positive effects. The studies by Xiao et al. (2013), Lambert et al. (2016) and Bañuls et al. (2015) also all showed improvements in insulin sensitivity.

There is evidence of a causal relationship between insulin resistance and weight gain from classical studies of experimental overfeeding in which lean individuals with no history of obesity or diabetes became insulin resistant (Sims and Danforth, 1987). There is also a considerable amount of literature published linking obesity with type 2 diabetes usually from a causal standpoint (Kahn and Flier, 2000; Martyn, Kaneki, and Yasuhara, 2008; Hardy, Czech, and Corvera, 2012). The results of this present study suggest that the gut microbiota can positively influence blood glucose levels and interventions that modify the microbiota may be useful in treating obesity by

either helping in weight loss or at least by lowering the risks of associated diseases such as type 2 diabetes and MetS. These results highlight the importance of the need for further research into the understanding of the links between hyperglycaemia and insulin resistance in obesity.

Although no changes were seen in the ratio of Firmicutes to Bacteroidetes in the study by Xiao et al. (2013) it appears that overall this particular study was well designed, measured the appropriate outcomes and had potentially beneficial results. The use of diet including foods rich in dietary fibre with the addition of prebiotics including resistant starch and oligosaccharides seems to be the best approach when designing trials to investigate the potential of diet in modulating the gut microbiota. Xiao et al. (2013) do caution that due to the self-controlled nature of their study it was not possible to definitively conclude that the results were due to modification of the gut microbiome. It is possible that the changes in measures could, to a greater or lesser degree be as a result of the reduction in body weight or from the changes in nutrients from the prescribed diet rather than via the changes in gut microbiota (Xiao et al. 2013). They do, however, suggest that the changes in composition were of a beneficial nature due to the protective qualities of certain microbiota, which supports the findings of Shanahan (2002), Griffiths et al. (2004) and (Sekirov et al., 2010).

The studies by Remely et al. (2015) and Simões et al. (2013) that used diet only reported beneficial changes to the gut microbiota including reductions in the ratio of Firmicutes to Bacteroidetes and increases in Lactobacilli (Remely et al. 2015) and Bifidobacteria (Remely et al. 2015; Simões et al. 2013).

These studies support in part the findings of Xiao et al. (2013) in relation to the protective actions of certain microbiota on the epithelial layer of the gut lining. The studies that used prebiotic supplementation on a whole showed little effect on the gut microbiota, however, there was some evidence that the prebiotics had a beneficial effect on the reduction of body fat and glucose tolerance (Lambert et al. 2016; Dewulf et al. 2012). Lappi et al. (2013) suggests that the act of modulating the gut microbiota by only using a prebiotic in the form of indigestible carbohydrate is not straightforward. This is because the pre-existing composition of the microbiota as well as other

dietary components such as fatty acids can have an effect on the outcome. It is evident that prebiotic supplementation can be beneficial, however more research is needed to test the different types of prebiotics including resistant starches, oligosaccharides, and plant fibres to ascertain effective doses. The studies would benefit from measuring biomarkers relating to MetS, including glucose levels, insulin resistance inflammation as well as weight reduction. Some form of standardisation is needed in regard to the diet of participants during the study period to limit the number of confounders. Although dietary fat and protein have been shown to influence the microbiota none of the included studies looked specifically at the effect of these nutrients choosing instead to focus on carbohydrate prebiotics. Future studies would benefit in examining the effects of diets containing different levels of fat, protein and carbohydrate to establish what ratio of macronutrients is most beneficial in creating a diverse and healthy gut microbiome.

Only three of the studies (Xiao et al. 2013; Lambert et al. 2016; Bañuls et al. 2015) mention limitations. Xiao et al. (2013) suggest that the self-controlled nature of their study limited it to some degree as it was not possible to know if all the effects shown were due to the manipulation of the gut microbiota or some other factor. This can also be said about the studies of Remely et al. (2015) and Simões et al. (2013) which were also self-controlled. Self-controlled, non-randomised interventions can, however, be useful in situations where randomization is not practical and often allow for a broader inclusion criteria than randomized trials and can yield a study population that can be more representative of any given target population (Velengtas, Mohr, and Messner, 2012).

Although the study by Lambert et al. (2016) was randomised, double blind and placebo controlled the authors acknowledge that the participants were on self-selected diets meaning there was no control over calories or macronutrients. It would therefore not be possible to determine whether the diet of the participants had a limiting effect on the prebiotic supplement. Similarly the study by Dewulf et al. (2012) was double blinded and placebo

controlled but again the diets of the participants were not controlled. In these interventions there was very little effect on body composition. Bañuls et al. (2015) conceded that they were unable to confirm whether the carbohydrate metabolism response was due to the L- carnitine supplementation or the dietary fibres due to the fact that the two compounds were combined. Another weakness of the Bañuls et al. (2015) was the sample size, however, all of the studies were of a similar size except Simões et al. (2013) which was smaller with 16 participants. It is possible that with larger numbers of participants the results from the studies may have been more significant. A limitation of all the studies involving prebiotics only was their relatively short duration, the longest being Dewulf et al. (2012) with 13 weeks. Many of the benefits shown in the diet and diet plus prebiotic studies only happened or became significant in the later stages of the interventions which may suggest that given longer similar changes would have occurred in the prebiotic only studies. Therefore, the duration of future studies should be taken into account to ascertain the shortest time at which positive results are to be expected. The limitations stated here to a lesser or greater degree explain why this review was not able to provide a definitive conclusion as to the effectiveness of the dietary interventions aimed at modifying the gut microbiota to reduce obesity and highlight why more research is needed.

As mentioned previously Harley and Karp (2012) proposed three mechanisms for how the microbiota may cause weight increase leading to obesity. It appears that not all the studies support these mechanisms and therefore do not show a causal link between the microbiota and obesity. To discover any causal links future research would benefit from being of robust design and methodology. The studies should have a control group and be randomised for the reasons stated previously. The interventions should be of a duration that allows the effects of any changes in microbial composition to be observed. Body composition as well as biomarkers for MetS need to be measured along-side analysis of the gut microbiota composition. Correlation analysis to evaluate any potential links between changes in gut microbiota composition brought about by prebiotics and diet should be included.

According to Nagpal, Yadav, and Marotta (2014) for the therapeutic benefits to be fully realised, the structure, density, and roles of the gut microbiota need to be comprehensively understood. Only then will it be possible to know how interventions can be used by clinicians, nutritionists, dietitians and health care professionals to reduce the prevalence of obesity and its comorbidities.

According to Xiao et al. (2013) using dietary interventions to reduce the endotoxin load caused by gut dysbiosis from the over growth of certain strains of bacteria that leads to inflammation looks to be a potentially promising method for reducing obesity and related metabolic disorders. This view is supported directly or indirectly by Remely et al. (2015), Simões et al. (2013), Dewulf et al. (2012) and Bañuls et al. (2015) who have all demonstrated either weight loss or beneficial changes in the composition of the gut microbiota related to reducing inflammation or improving blood glucose/ insulin sensitivity.

A possible limitation of this present study was the relatively short time frame in which the study was completed. This may have had an impact on the number of articles that could be searched comprehensively enough to make decisions relating to the inclusion or exclusion of relevant studies. Time was also a factor in researching the search terms that were used for the main search. Choosing only human trials limited the number of studies included, however, it was decided that the purpose of this study was to examine dietary interventions for humans and due to the limitations discussed previously all animal studies were excluded.

6 Conclusion

Although this review was unable to arrive at a definitive conclusion it has identified several lines of evidence for the potential of dietary interventions aimed at reducing the prevalence of obesity and its comorbidities through modification of the gut microbiota. The potential benefits of dietary interventions are brought about by the protective actions of certain strains of the gut microbiota on the epithelial layer of the gut preventing leaky gut and the translocation of certain types of bacteria and endotoxins. It is also

possible that modifying the composition of the major phyla may be able to balance energy extraction and utilisation. However, further research is needed to fully understand the mechanisms and possible causal relationships between the gut microbiome and obesity. Intervention studies in humans using diet and/or prebiotic formulas have been valuable in determining the relevance of certain bacteria in relation to obesity. So far there has been a failure to establish a causal link with much of the evidence being controversial. Studies that modulate specific microbiota through supplementing with prebiotics, observing the microbiota's response and the subsequent effects on an individual's health may help to establish this link.

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