STRAWBERRIES AND GUT HEALTH IN POSTMENOPAUSAL WOMEN

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Morgan T. MacNeill

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TITLE: Strawberries and Gut Health in Postmenopausal Women

AUTHOR: Morgan T. MacNeill

DATE SUBMITTED: June 2019

COMMITTEE CHAIR: Kari D. Pilolla, PhD, RD
Assistant Professor of Food Science and Nutrition

COMMITTEE MEMBER: Michael La Frano, PhD, RD
Assistant Professor of Food Science and Nutrition

COMMITTEE MEMBER: Hunter Glanz, PhD
Assistant Professor of Statistics
ABSTRACT

Strawberries and Gut Health in Postmenopausal Women

Morgan T. MacNeill

The gut microbiota has been implicated in both health and disease. As such, diet is a significant determinant of gut health, whereby diet induced dysbiosis is associated with cardiometabolic risk. Interestingly, a higher proportion of Firmicutes and a lower proportion of Bacteroidetes are implicated in obesity. Strawberry polyphenols have been shown to reduce cardiovascular disease risk in addition to exhibiting prebiotic activity by increasing probiotic bacteria in the gut. Polyphenols have also been shown to reduce the ratio of Firmicutes to Bacteroidetes. Therefore, dietary modifications such as strawberry consumption may help improve health outcomes through the gut. The objective of this study was to analyze whether 13 g freeze dried strawberry powder (~1 cup/d fresh) consumption reduces the Firmicutes:Bacteroidetes ratio and increases microbial diversity and beneficial bacteria like Lactobacillus and Bifidobacterium. This study was a 5-week free-living diet intervention trial conducted at California Polytechnic State University, San Luis Obispo and The Eye Medical Center of Fresno. Participants (n=10) had a mean age of 60.5 ± 9.13 years and had a mean body weight of 74.71 ± 10.61 kg. The participants completed a 3-week washout before a 2-week diet intervention. Participants maintained their normal diet throughout the study while eliminating foods high in polyphenols and probiotics. Upon completion of the study, no significant differences were found for body weight (p=0.22) or BMI (p=0.26). Likewise, no significant differences were found for macronutrient, vitamin, or mineral intake except for sugar (p=0.03), vitamin B12 (p=0.03), and fruit (p=0.0014). Bacteria abundance and diversity were not found to be statistically significant following intervention. Since strawberry supplementation was not associated with a significant change in the relative abundance of bacteria with the dose and duration administered, a randomized controlled trial would better determine the effect of strawberry consumption on gut health.
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1. INTRODUCTION

Recent research has identified an association between the gut microbiota and overall health. Known factors such as genetics and lifestyle choices are shown to influence the composition of the gut microbiota. Specifically, research has shown a significant relationship between diet and the gut microbiota.\(^1\) Diet affects a variety of variables related to gut health. For instance, diet-induced dysbiosis (imbalance of healthy and harmful gut bacteria) has been linked to atherosclerosis, obesity, and type 2 diabetes.\(^2\)

Additionally, research has demonstrated the association between the ratio of the phyla Firmicutes and Bacteroidetes and specific health outcomes. For example, an increased Firmicutes:Bacteroidetes (F:B) ratio potentially contributes to adiposity through greater energy harvest and activation of lipopolysaccharide (LPS) accompanied by changes in the intestinal barrier integrity.\(^3\)

Recent literature has also identified a relationship between sex hormones, the gut microbiota, and their link to various disease states. Specifically, postmenopausal women see a decline in estrogen as they begin menopause\(^4\) which is accompanied by an increased risk for CVD.\(^5\) Research has found that CVD risk may be related to the gut microbiota’s ability to process estrogen.\(^6\) Lifestyle choices like diet may exacerbate this process by contributing to a state of dysbiosis, and therefore, resulting in decreased ability to metabolize estrogen.\(^6\) As it stands, postmenopausal women have a high Firmicutes:Bacteroidetes ratio compared to men, and reduced baseline short chain fatty acid (SCFA) metabolism, both of which may also contribute to the pathogenesis of CVD.\(^7\)
As such, diet, especially fruit intake, may be an effective approach to improve health outcomes through the gut. Namely, polyphenols found in strawberries have been reported to stimulate growth of commensal and probiotic bacteria while selectively inhibiting pathogen growth.\textsuperscript{8}

Previous diet intervention trials have investigated the consumption of various high polyphenol fruit, berry polyphenols, and berries on the human gut microbiota profile\textsuperscript{9-19} whereas studies that assess the impact of strawberry consumption on the gut have yet to be investigated. The effect of strawberry consumption on the composition and diversity of the gut microbiota in overweight postmenopausal women is currently unknown.

Given the documented negative impact of lifestyle choices, including diet, on gut health,\textsuperscript{1} and the potential for strawberry consumption to mitigate these effects, daily strawberry intake could be an alternative to expensive treatment methods that can generate unwanted side-effects. Thus, the objective of this study is to assess and determine the effect of strawberry consumption on the diversity and composition of the gut microbiota. It is hypothesized that daily consumption of 13 g (~1 cup fresh strawberries) freeze-dried strawberry powder will reduce the ratio of Firmicutes to Bacteroidetes while also increasing the microbial diversity and the abundance of several probiotic bacteria including Bifidobacterium and Lactobacillus.
2. LITERATURE REVIEW

2.1 Gut Microbiota in Healthy Individuals

The bacteria that inhabit the gut have the potential to influence overall human health and well-being. Gut microbes produce large numbers of bioactive compounds, including vitamins and short-chain fatty acids (SCFAs), that promote cellular mechanisms which maintain tissue integrity.\(^1\) However, while a large majority of the bacteria are innocuous, they may also play a role in chronic diseases.\(^20\) Recent findings have consistently observed that low bacterial diversity is associated with different diseases and health conditions including obesity and intestinal inflammation.\(^21\) As such, the exact role of the gut microbiota in the onset of disease is still being explored. That is, does disease precede changes to the microbiota composition or do changes in the gut composition lead to disease. Nonetheless, microbial diversity has been linked with the metabolic functions of the gut bacteria, and thus has the potential to influence human health.\(^21\)

2.1.1 Composition of the human gut microbiota

The human gastrointestinal tract represents a large microbial ecosystem, housing several trillion microbial cells, specifically bacteria.\(^22\) To date, there have been over 50 bacterial phyla identified,\(^23\) with Bacteroidetes and Firmicutes representing approximately 90% of the gut microbiota\(^24\); Proteobacteria, Actinobacteria, Verrucomicrobia, and Fusobacteria exist in smaller proportions (Figure 1).\(^24\)
Figure 1. Predominant taxonomic gut microbiota composition, Rinninella, 2019.\textsuperscript{24}
2.1.1.1 Predominant gut bacteria and their functions

2.1.1.1.1 Bacteroidetes

The Bacteroidetes phylum make up ~23% of the gut composition\(^{25}\) and includes genera known for their role in human health. Bacteroides (approximately 75% of the Bacteroidetes phylum\(^{26}\)) and Prevotella are two genera within the Bacteroidetes phylum which specialize in the metabolic conversion of protein and complex carbohydrates (i.e. plant polysaccharides like cellulose, starch, pectins, and xylans) to their respective metabolites. In addition, the Bacteroidetes phylum are major producers of the SCFA propionate\(^{27}\) while some Bacteroides spp. deconjugate bile acids.\(^{28}\)

2.1.1.1.2 Firmicutes

The Firmicutes phylum constitutes ~50%-80% of the gut microbiota.\(^{28}\) Notable genera include Clostridium, Lactobacillus, Bacillus, Enterococcus, and Ruminicoccus; Clostridium represents 95% of the Firmicutes phylum.\(^{24}\) Most butyrate productions occurs in the Firmicutes phylum\(^{29}\) with several microbial communities capable of fermenting carbohydrates to lactate.\(^{28}\) For instance, Streptococcus spp. ferment simple sugars into lactate, and the lactate is converted into propionate by Veillonella spp.\(^{28}\) Likewise, the Lactobacillus spp. produce lactic acid from carbohydrate fermentation and Ruminococcus spp. degrade resistant starch to produce acetate.\(^{28}\)

2.1.1.1.3 Actinobacteria

Though the Bacteroidetes and Firmicutes phyla dominate the gut microbiota, there are some phyla present in smaller quantities that play a significant role in human health. The Actinobacteria phylum composes ~3% of the gut microbiota\(^{25}\) and is dominated by
the Bifidobacterium genus.\textsuperscript{29} Bifidobacterium spp. are predominant in the infant gut (90% of total microbiota), and their abundance declines to <5% in adults.\textsuperscript{29} This genus contributes to gut health by producing lactate and acetate through carbohydrate fermentation.\textsuperscript{29} Bifidobacterium ferment non-digestible carbohydrates including resistant starch, pectin, inulin, cellulose as well as carbohydrates like mucin and human milk oligosaccharides produced by the host.\textsuperscript{29} In addition, Bifidobacterium can produce vitamin B12 and defensive bacteriocins.\textsuperscript{29} Other phyla represented in Figure 1 (i.e. Proteobacteria; Fusobacteria; Verucomicrobia) will not be detailed as they are not phyla and/or include genera that directly relate to the study objectives (objectives are discussed in section 3.1).

\textbf{2.1.2 Functions of the human gut microbiota}

Through ongoing research, it is recognized that gut microbial communities function like an organ that benefit both the host and the bacteria.\textsuperscript{30} Collectively, the functions of the gut microbiota can be broken down into three categories: metabolism, biosynthesis, and effect on the intestinal environment. As such, the gut microbiota are critical in the daily functioning of the human body by degrading non-digestible food compounds, synthesizing essential vitamins and SCFAs and assisting in producing metabolic end-products.\textsuperscript{29} The microbiota also stimulate the host immune system to produce defensive agents against harmful bacteria, and therefore maintain a favorable environment for native commensal bacteria.\textsuperscript{31}
2.1.2.1 Colonic Metabolism

The gut microbiota play a significant role in the digestion and colonic metabolism of food compounds, including dietary nutrients and phytochemicals. For example, \textit{Bacteroides thetaiotamicron} produces a collection of enzymes in a multi-step degradation of carbohydrates.\textsuperscript{1} In addition, bacterial phytases can degrade phytic acid in grains which release minerals including calcium, magnesium, and phosphate.\textsuperscript{1} Additionally, degradation of the polysaccharide and protein rich mucus layer allow bacteria to meet their own energy needs while assisting in the turnover of the mucus layer.\textsuperscript{1} Establishing a healthy mucus layer has been found to maintain endothelial integrity, therefore, preventing potentially harmful gut conditions such as endotoxemia.\textsuperscript{1}

2.1.2.2 Bile acid metabolism

Bile acids are needed to facilitate the absorption of fat, cholesterol, and fat-soluble vitamins from the intestine. The bile acids that do not recirculate to the liver are de-conjugated by gut bacterial bile salt hydrolases (BSH), generating secondary bile acids.\textsuperscript{32} De-conjugation reactions including dihydroxylation, dehydrogenation, and epimerization are performed by, but not limited to, the genera Bacteroides, Clostridium, and Eubacterium.\textsuperscript{33} Recent literature has determined an association between bile salt hydrolase activity and control of obesity and hypercholesterolemia. Joyce et al. found that by elevating BSH, it reduced weight gain, serum cholesterol, and liver triglycerides by directing expression of signaling pathways known for their role in lipid metabolism, circadian rhythm, and epithelial cell function.\textsuperscript{34}
2.1.2.3 Biosynthesis

2.1.2.3.1 Vitamin production

A well-documented function of the gut microbiota is its role in the biosynthesis of vitamins. Gut bacteria like Actinobacteria and Bacteroidetes can generate vitamin K and B group vitamins including thiamin, biotin, cobalamin, niacin, pyridoxine, folate and vitamin B₁₂.³⁵ Intestinal production of several vitamins individually contributes to a quarter or more of the suggested daily reference intake. For instance, production of folate, niacin, pyridoxine, and cobalamin reach 37, 27, 86, and 31 percent of the suggested dietary intake respectively.³⁵ These vitamins participate in numerous metabolic reactions throughout the body, with significant roles in blood clotting, hematopoiesis, and tissue repair which maintain healthy nervous and cardiovascular systems.³⁶

2.1.2.3.2 Short Chain Fatty Acid production

One of the most physiologically important products of the gut microbiota are the SCFAs produced by microbial fermentation of non-digestible dietary fiber.¹ SCFAs provide energy for colorectal tissues and bacteria and promote cellular mechanisms that encourage tissue integrity.¹ The SCFAs consist of acetate, butyrate, and propionate which collectively contribute to host health through various processes.³⁷

All three SCFAs can decrease pH in the colon which deters pathogen growth. Specifically, acetate increases blood flow and oxygen uptake in the colon, acts as a co-substrate to produce butyrate, and once absorbed, is an energy source for muscle and brain tissue.²⁹ Propionate prevents proliferation of and induces apoptosis of colorectal cancer cells,
interacts with the host immune system, promotes satiety, lowers blood cholesterol levels and improves insulin sensitivity.\textsuperscript{29} In a human study, inulin-propionate ester significantly increased postprandial plasma PYY and GLP-1, and over the course of 24 days, propionate supplementation significantly reduced weight gain, intra-abdominal adipose tissue distribution, and intrahepatocellular lipid content. Propionate also prevented the decline in insulin sensitivity that was observed in the inulin-control group.\textsuperscript{38} Epidemiological evidence also suggests that propionate can travel through the circulatory system to impact immune function and inflammation in peripheral tissues such as the lung.\textsuperscript{1} Lastly, butyrate stimulates the absorption of water and sodium in the colon, reduces oxidative stress, prevents colon cancer and colitis, and improves gut barrier function by stimulating mucin formation, antimicrobial peptides, and tight-junction proteins.\textsuperscript{29} These effects may reduce the likelihood of endotoxemia should any pro-inflammatory substances leak across the gut barrier.\textsuperscript{39} Butyrate also acts to increase host insulin sensitivity by stimulating the release of gastric inhibitory polypeptide from enteroendocrine K-cells.\textsuperscript{39} Metabolically, both butyrate and propionate can regulate energy intake, expenditure, and storage by stimulating the release of the satiety hormones glucagon-like peptide 1 (GLP-1) and peptide YY from enteroendocrine L-cells, therefore encouraging satiation.\textsuperscript{38,39}

\textbf{2.1.2.4 Effect on the intestinal environment}

Another vital role of the gut microbiota is protecting against pathogen colonization and maintaining a healthy gut environment.\textsuperscript{31} The microbiota achieve this homeostasis
through competitive metabolic interaction, recruiting host immune responses, and encouraging vascularization.

The gram negative and gram positive native commensal bacteria deter pathogen growth by producing bacteriocins and proteinaceous toxins that inhibit members of the same bacterial species. For instance, E. coli can produce bacteriocins when it needs to fend off the related pathogen enterohaemorrhagic E. coli. Commensal bacteria and SCFAs can also alter the pH of the gut environment to a level that prohibits pathogen colonization. This allows the commensal bacteria to occupy intestinal niches as colonization sites that could otherwise be filled by pathogenic bacteria.

Additionally, the commensal bacteria fend off pathogens and encourage epithelial integrity by communicating with the host immune system. Since the lining of the gut is the largest surface area in contact with exogenous antigens, the gut microbiota play a central role in mucosal immunity and potentially preventing bacterial translocation. Research shows that the commensal bacteria promote epithelial barrier function by synthesizing antimicrobial peptides resulting in fewer scenarios of pathogen translocation. Over time, the presence of commensal bacteria may result in decreased incidence of pathogen associated disease.

Research has also investigated the role of the intestinal bacteria in vascularization. Stappenbeck et al. compared germ-free mice and B. thetaiotaomicron-colonized transgenic mice with Paneth cells and found that the bacteria shaped the development of the intestinal villus microvasculature through Paneth cell dependent interaction.
This study emphasizes how the gut microbiota may better promote absorption of nutrients through increased vascularization.

2.1.3 Gut associated disease states

Through their many functions, gut bacteria have the capacity to help or harm the human body. Small disturbances to their environment, and therefore the gut ecology, can result in systemic complications for the human host. As such, dysbiosis and low diversity have been associated with various disease states.²

The diversity of the gut microbiota has been associated with human health.⁴¹ A healthy gut microbiota is characterized by high diversity with the ability to resist change under stress, while lower species diversity and fewer beneficial microbes and/or presence of pathobionts are associated with disease.² Gut microbial diversity, measured via intestinal biopsies or fecal samples, is the number and abundance of distinct types of organisms found in the gastrointestinal tract and can be defined three ways.⁴² Alpha diversity is the average species diversity in a habitat; beta diversity is the diversity of species between two habitats; gamma diversity is the total diversity of a landscape, and is the combination of alpha and beta diversity.⁴³

Wong suggests that one advantage of having a greater microbial diversity could be to guarantee that metabolic functions are unaffected by changes in gut composition, whereby select microbes with similar functions can fill in for other microbes when a certain metabolic task needs to be performed.⁴¹ Valdes et al. proposed that diversity is a
good indicator of a ‘healthy gut’ in the sense that a diverse bacterial ecosystem will compensate for missing species.\textsuperscript{44}

When the composition of the gut microbiome is altered, such as a reduced diversity, a state of dysbiosis is present, or an imbalance of helpful and harmful bacteria.\textsuperscript{44} Low diversity can reduce resistance to pathogenic bacteria colonization, resulting in the expansion of harmful bacteria.\textsuperscript{45} Low diversity may also limit production of SCFAs since less bacteria of different types are available for fermentation.\textsuperscript{46} This dysbiosis may form the basis for the pathogenesis of disorders such as atherosclerosis, IBS, diabetes, and obesity.\textsuperscript{2} Notably, the imbalance of Firmicutes and Bacteroidetes has been a point of interest in gut research as varying levels of their abundance is associated with several disease states,\textsuperscript{47,48} namely obesity. The ratio of Firmicutes to Bacteroidetes in healthy infants, adults, and elderly are reported to be 0.4, 10.9, and 0.6 respectively.\textsuperscript{49} Conversely, the F:B ratio varies among obese and lean individuals, with some studies reporting an increased F:B ratio in obesity,\textsuperscript{48,50,51} while others report the opposite relationship.\textsuperscript{52} Still, other studies have not found a correlation between BMI and the reported F:B ratio.\textsuperscript{53} While the ratio of F:B has been quantified in healthy populations, a taxonomic signature has yet to be established for unhealthy populations\textsuperscript{53} due to interindividual variability from differences in diet, lifestyle, and other factors.

\subsection{Atherosclerosis}

Dysbiosis has been identified as a strong risk factor for atherosclerosis, specifically through the production of trimethylamine-N-oxide (TMAO).\textsuperscript{22} TMAO inhibits reverse cholesterol transport and is formed from trimethylamine (TMA) which is a product of
gut microbial degradation of dietary precursors like l-carnitine and phosphatidylcholine.\textsuperscript{54} TMA is converted into TMAO in the liver by hepatic flavin monooxygenase 3.\textsuperscript{55} The gut microbiota that are thought to be involved in the initial conversion of l-carnitine and phosphatidylcholine to TMA include genera from the Firmicutes and Proteobacteria phyla including Clostridium spp.,Escherichia fergusonii, and Edwardsiella tarda.\textsuperscript{55} Furthermore, foods high in levels of l-carnitine and phosphatidylcholine, such as cheese, seafood, eggs, and red meat, can accelerate the development of atherosclerosis through microbial TMAO production.\textsuperscript{46} Gut microbiota-mediated therapy has been proposed as one strategy to initiate inhibition of microbial TMAO synthesis.\textsuperscript{22} In this way, the gut microbiota behaves as a potential preventive agent of disease.

\subsection*{2.1.3.2 Type 1 and Type 2 Diabetes}

The gut microbiota has also been implicated in other metabolic diseases, specifically diabetes. A study by Larsen et al. compared the composition of the intestinal microbiota in type 2 diabetics versus non-diabetics.\textsuperscript{47} The results found a significantly reduced (p=0.03) abundance of Firmicutes in the diabetic group (36.8\% mean) compared to controls (56.4\%), while Bacteroidetes was increased but not significantly in the diabetic group.\textsuperscript{47} Similar results were captured in a study comparing children with type 1 diabetes to healthy children. The ratio of F:B in diabetic children (0.62) was significantly lower (p=0.001) than in healthy children (0.97).\textsuperscript{56} Both studies found that the F:B ratio correlated negatively and significantly to plasma glucose level and concluded that this ratio could be implicated in the glycemic level of the diabetic individuals.
2.1.3.3 Irritable Bowel Syndrome

Altered gut communities are also seen in irritable bowel syndrome (IBS). In a review, Collins et al. discussed a variety of studies that repeatedly indicated an association between IBS, bacterial dysbiosis, and altered ratios of bacteria species.\(^{57}\) For example, when germ-free animals were colonized with fecal bacteria from patients with IBS compared to healthy controls, it resulted in maintenance of IBS symptoms in the germ-free animal. The microbial dysbiosis of the IBS gut microbiota (i.e. more sulfate-reducing bacteria and less Bifidobacterium) along with hypersensitivity to colonic distension were maintained.\(^{58}\) In addition, several studies have seen an increase in the phylum Firmicutes and a decrease in the genus Bacteroides in IBS patients.\(^{57}\) Interestingly, triggers such as infection, stress, and antibiotic use initiate dysbiosis, which can alter the gut microbiota and may account for the characteristic symptoms of IBS over time.\(^{57}\)

Presence of IBS has implications for overall health as the condition may impact absorption of nutrients from the diet such that when the gut microbiota is disturbed, the body may become less efficient at converting food to usable products.

2.1.3.4 Obesity

Alterations in the human gut microbiota has also been identified as a risk factor for obesity, however, there is debate as to what capacity the gut microbiota contributes to the pathophysiology of obesity. That is, does obesity result from changes in the gut microbiota or does an obese status alter the gut microbiota? Nevertheless, several mechanisms have been proposed to account for this observation: (1) increased energy
harvested from the diet; (2) and changes in the intestinal barrier integrity linked to lipopolysaccharide (LPS).³

In general, research indicates that there may be an association between the efficiency of the gut microbiota to extract energy from the diet and the development of obesity. Turnbaugh et. al. tested the mechanism behind this observation and found that when an obese microbiota was colonized into germ-free mice, it resulted in a significantly greater increase in total body fat.⁵⁹ An increased concentration of butyrate and acetate were also seen in the gut, which was accompanied by significantly less energy remaining in their stools relative to the lean controls. Further, the obese microbiome had a substantial increase in genes that encoded enzymes involved in the breakdown of dietary polysaccharides.⁵⁹ A suggested mechanism that linked the gut microbiota to this observation included provision of additional energy via conversion of dietary fiber to SCFAs.⁵⁹

Further, a human energy balance study investigated how diets that varied in caloric content impact the gut composition. Researchers found that alteration of the nutrient load (2400 kcal to 3400 kcal) resulted in rapid changes in the gut microbiota. A 20% increase in Firmicutes was associated with an increased energy harvest of ~150kcal and a 20% increase in Bacteroides was associated with a decreased energy harvest of ~150kcal, suggesting the gut microbiota’s role in regulation of nutrient harvest.⁶⁰

Another proposed mechanism that links the gut microbiota to obesity is the presence of gut microbiota derived LPS.⁶¹ LPS is an inflammatory cell wall constituent of Gram-
negative bacteria that, when released due to cell division or death, can trigger an inflammatory cascade through Toll-like receptor-4 (TLR4), CD14, or NF-κB. Concerning obesity, a hypothesis is that when LPS leaks into circulation, TLR4 activates pro-inflammatory pathways where cytokine expression induces altered metabolic function in adipose tissue. Evidence of this interaction has been explored in animals and humans. For example, infusion of LPS increased adipose tissue, insulinenia, and liver insulin resistance in mice. Additionally, in women, intestinal permeability correlated with visceral adiposity which was proposed to be related to LPS. Furthermore, a positive correlation between serum LPS and BMI, high triglycerides, and central adiposity was seen in young obese subjects. Together, these findings establish the possible relationship between LPS and obesity. However, since there remain questions relating the gut microbiota to health outcomes, it becomes increasingly more important to assess how both non-modifiable and modifiable factors like diet can alter the gut composition.

2.1.4 Factors that influence the gut microbiota composition

As previously mentioned, the gut microbiota seems to behave like a fluid ‘organ’ that continuously adapts to its environment. As such, there are non-modifiable factors like genetics, age, and hormones and modifiable factors like antibiotics, smoking, exercise, and diet that contribute to its composition and associated functions.
2.1.4.1 Non-modifiable factors that influence the gut microbiota

2.1.4.1.1 Genetics

While there is intraindividual variability in microbial communities, the human microbiota is generally stable at the phylum level with variation in phylum proportions between individuals.\(^{23}\) As such, genetic factors can govern these individual differences seen in the microbial populations. In a metagenomic study, researchers compared twin pair microbiotas across 1,000 fecal samples from the TwinsUK population. The study identified a variety of microbial taxa whose abundance was influenced by host genetics,\(^{65}\) indicating a link between host genetics and the gut microbiota. Nevertheless, the Bacteroidetes community was found to be shaped mostly by environmental factors.\(^{65}\) This suggests that some bacterial species are not heritable and are likely influenced by other factors.

2.1.4.1.2 Age

In addition to genetics, the composition of the microbiota changes with age (Figure 2). Microbes begin to colonize the gut shortly after birth and the bacteria continue to develop during breastfeeding as the oligosaccharides in breast milk encourage growth of Lactobacillus and Bifidobacterium.\(^{1}\) Once the baby switches to whole foods, the bacteria population shifts to favor bacteria that are needed to utilize fiber and other nutrients present in adult diets like Bacteroidetes and Firmicutes.\(^{1}\) While a variety of factors govern the composition of the gut microbiota—including genetics, puberty, ovarian cycle, pregnancy, and menopause – age is independently associated with the abundance of particular bacteria.\(^{1}\)
The gut microbiota of infants (3wk-10mo), adults (25-45yr), and older adult (70-90yr) populations were sequenced with the following results: the infant gut microbiota was dominated by Bifidobacterium (Actinobacteria phylum), the adult gut microbiota was dominated by Firmicutes and Bacteroidetes, and the gut microbiota of older adults was dominated by Bacteroidetes and Firmicutes with a significant presence of E. coli compared to adults. The Bacteroides genus abundance was equivalent in all age groups. Total bacteria count was significantly lower in infants than in adults and seniors. Regarding the elderly population, the gut microbiota of 17 individuals from a geriatric department showed that the proportion of Bacteroidetes was significantly higher than in younger adults with similar findings reported by Claesson et al.

Reasons for the shifts seen in dominant bacterial species are unclear, but living situation (i.e. long-term care vs. community dwelling), altered diet, changes in digestive physiology, and reduction in transit time and digestive secretions have been postulated.
2.1.4.1.3 Interaction between sex-related hormones and the gut microbiota

In addition to age, a person’s sex may influence the gut microbiota. Considering the age-related decline in sex hormones in both men and women, researchers have investigated the relationship between estrogen and the gut microbiota. Santos-Marcos et al. analyzed the gut microbiota in 17 premenopausal and 20 postmenopausal women and matched the two groups with men by age. Results showed a higher Firmicutes abundance in postmenopausal women versus premenopausal women, with a higher F:B ratio in postmenopausal women versus men. In addition, estradiol levels positively correlated with various bacteria classes and families. Interestingly, the researchers observed a lower relative abundance of SCFA producing bacteria, with lower butyrate and propionate metabolism, in postmenopausal versus premenopausal women. This has implications for women’s health as SCFAs have been associated with metabolic
health. Teixeira et al. found that a higher level of fecal SCFA in women correlated with metabolic syndrome risk factors with the authors suggesting that increased colonic fermentation may contribute to obesity. However, SCFAs have also been shown to regulate metabolic homeostasis through AMP-activated protein kinase while reducing postprandial free fatty acids and increasing satiety hormones. Therefore, it is unclear whether SCFAs contribute to metabolic risk or have the opposite effect by regulating appetite and energy homeostasis. Nonetheless, the researchers concluded that the differences in gut composition between men and women were influenced by hormonal status in women, and these differences may influence incidence of metabolic disease and their varied prevalence in men and women.7

Interestingly, not only has estrogen been associated with the gut microbiota, but recent studies have shown that the gut microbiota is related to the development of CVD. Research indicates that a transfer of fecal microbiota induces metabolic disease and obesity. The literature also suggests that an association exists between metabolic risk and gut microbiota changes in postmenopausal women. In one study, fecal DNA from obese postmenopausal women were analyzed and a systematic search was performed for bacterial genes associated with markers of insulin resistance, inflammation, and lipid metabolism. Researchers found that 114 metagenomic species correlated positively or negatively with the previously mentioned metabolic markers. The authors also found that diet modulated beneficial bacteria and emphasized the importance of focusing on diet when studying the link between gut microbiota and metabolic markers.
As it relates to CVD risk in general, women tend to experience weight gain, particularly in visceral adipose tissue, a few years prior to menopause, with postmenopausal women experiencing greater intra-abdominal fat versus pre-menopausal women. This physiological change coincides with losing 80% of their estrogen per year beginning the first year of menopause which is accompanied by shifts in adipose tissue deposition and expansion. Deposits of fat, especially in visceral adipose tissue, correlates to increased circulating adipokines which are implicated in insulin resistance and CVD. As such, women exhibit larger risk for metabolic disease as they age and with the transition to postmenopausal status which is proposed to result from reductions in circulating estrogen.

As such, the association between estrogen and metabolic risk may be explained by its interaction with the gut microbiota. The gut microbiota secretes β-glucuronidase which convert estrogens to their deconjugated form. The estrogen then interacts with estrogen receptors to elicit downstream effects resulting in physiological changes in the uterus, ovaries, bone, breast, liver, muscle, white adipose tissue, and colon. In effect, the gut microbiota encourages estrogen homeostasis. If gut dysbiosis and low gut diversity occur, a reduction in estrogen metabolism is possible due to a lack of estrogen metabolizing bacteria. Furthermore, a dysfunction in these physiological responses could contribute to disease states including CVD, obesity, MetS, endometriosis, polycystic ovary syndrome, and breast cancer. Proposed mechanisms for some of these conditions include low gut microbiota diversity and low circulating estrogen levels.
Of interest, phytoestrogens may be able to reduce the risk of developing some of these conditions as they are a ligand for estrogen receptors. A recent article by Chen et al. explained that the microbiota can metabolize estrogen-like compounds to their active form, and these compounds can encourage proliferation of certain bacteria types. Phytoestrogenic foods, including soy and lignins, are also shown to improve weight gain and are associated with a lower rate of overweight and obesity. Thus, phytoestrogens may play a role in preventing MetS through gut transformation.

Furthermore, based on metagenomic analysis and the observed link between a decline in estrogen levels and metabolic health, postmenopausal women appear to be at an increased metabolic risk through both altered gut composition changes and the decline in estrogen that may be exacerbated by dysbiosis. Additionally, since a sedentary lifestyle, poor diet, and decreased mobility promote overweight and obesity, and combined with decreased estrogen levels in postmenopausal women, this age group becomes a target for gut dysbiosis, MetS, diabetes, and CVD.

2.1.4.2 Modifiable

Of the factors that influence the gut microbiota, there are a few that may be modified. Such factors include the use of medications, especially antibiotics, smoking, physical activity level, and diet.

2.1.4.2.1 Antibiotics

It has been well established that although antibiotics are critical for killing harmful bacteria, beneficial bacteria are often destroyed along with the harmful bacteria being
targeted by the antibiotics. Use of antibiotics can suppress the commensal microbiota community within the gut along with their resistance against pathogens. This provides an opportunity for pathogenic bacteria to colonize the gastrointestinal tract.\textsuperscript{31} One deadly complication that can arise from antibiotic treatment is Clostridium difficile associated diarrhea, resulting in decreased microbial diversity.\textsuperscript{23}

\textbf{2.1.4.2 Smoking}

Smoking has also been found to alter the gut microbiota. Capurso et al. found that smoking was associated with an increased rate of C. difficile infection while smoking cessation correlated with increased microbial diversity.\textsuperscript{77} The authors note, however, that confounders such as diet or an increase in body weight could have accounted for these changes.\textsuperscript{77}

\textbf{2.1.4.3 Exercise}

While antibiotics and smoking have been negatively associated with the composition of the gut microbiota, evidence supports the positive role of exercise in gut health. In a mouse model, Luo demonstrated that moderate exercise increased gene expression for antimicrobial peptides accompanied by a lower degree of intestinal permeability and bacterial translocation.\textsuperscript{78} Further, Mika et al. demonstrated that the onset of exercise increased Bacteroidetes and decreased Firmicutes, a ratio associated with leanness.\textsuperscript{79} Interestingly, O’Sullivan et al. explains that the vagus nerve controls gastrointestinal inflammation and exercise-induced activation of the nerve may encourage an anti-inflammatory environment in the gut.\textsuperscript{80} Therefore exercise may influence the quantity and quality of the gut microbiota composition.\textsuperscript{80}
2.1.4.2.4 Diet

The diet is among the most powerful influencers of the gut microbiota composition. The following section will detail its impact on the gut microbiota.

2.2 Dietary Effects on the Gut Microbiota

Due to the diverse and variable diet of the average human, the gut microbiota must also diversify to satisfy the body’s metabolic needs. As such, much of the microbial diversity in the human gut is due to the microbial enzymatic capacity required to degrade nutrients. For instance, adequate insoluble fiber and nitrogenous protein consumption encourage bacterial fermentation in order to produce SCFAs. Additionally, dietary intake appears to be a significant short- and long-term regulator of the composition of the gut microbiota. However, only a small number of randomized controlled dietary intervention trials have been conducted in humans, of which, diets rich in fiber, fruit, and vegetables are associated with gut microbial activity that are linked to health benefits including increased abundance of probiotic bacteria and decreased intestinal inflammation. Thus, research seeks to isolate specific dietary patterns that increase microbial diversity while discouraging dysbiosis.

2.2.1 Flexibility of the gut composition

Diet can selectively and quickly alter the gut microbiota composition within days. One of the few studies in this area was by David et. al. who demonstrated how the gut microbiota can be rapidly altered by diet. For five consecutive days each, participants consumed two diets: a ‘plant-based diet’ rich in grains, legumes, fruits, and vegetables; and an ‘animal-based diet’ rich in meats, eggs, and cheeses. The animal diet observed a
significant increase in β-diversity after a single day but the gut microbiota reverted to their original structure two days after the diet intervention ended.\textsuperscript{81} This study showed that the human gut microbiota can rapidly switch between herbivorous and carnivorous bacterial profiles in order to maximize nutrient utilization.

2.2.2 Diet-induced microbial diversity

Diversity has been associated with different diet patterns. For instance, individuals consuming a plant-based diet versus a meat-based diet are shown to have a more diverse fecal microbiota composition.\textsuperscript{1} In fact, the phylogenetic diversity seen in the human gut is as follows: herbivore > omnivore > carnivore.\textsuperscript{82} Consuming a complex diet may increase levels of different types of bacteria and therefore increase SCFA production.\textsuperscript{46} With these considerations, if a diet-induced imbalance occurred, the microbiota can adapt, and the host will be less susceptible to disease and more resilient to stress.\textsuperscript{2}

2.2.3 Diet-induced microbial dysbiosis

Normally, the gut microbial communities are in symbiosis with the host and perform their physiological functions. However, diet can lead to microbial dysbiosis in the gut. Diet-induced dysbiosis is associated with disturbed gut barrier functions, increased gut permeability, and increased plasma LPS concentrations, leading to low-grade inflammation that is associated with diseases such as obesity and MetS.\textsuperscript{29,62,83}

Regarding diet-induced dysbiosis, consuming excess dietary fat is shown to expose the body to potentially pro-inflammatory free fatty acids which can alter the gut
composition and increase plasma LPS.\textsuperscript{61} Mice fed a high-fat diet saw increased plasma LPS concentration by favoring the growth of certain Gram-negative bacteria resulting in increased liberation of LPS.\textsuperscript{62} Dysbiosis from decimation of Firmicutes and Bacteroidetes was associated with a disrupted intestinal barrier and LPS leakage across the gut wall due to reduced tight junctions or carried with fat that was absorbed from the gut.\textsuperscript{62}

### 2.2.4 Diet, Obesity, and the Firmicutes:Bacteroidetes ratio

As mentioned previously, the human gut microbiota is composed of 50-80% Firmicutes and ~23% Bacteroidetes. An enlarged Firmicutes and reduced Bacteroidetes ratio (F:B) seem to represent the ‘bacterial trademark’ that characterizes obesity.\textsuperscript{90} As such, human and animal data support the theory that an increased ratio of Firmicutes to Bacteroidetes may contribute to the pathophysiology of obesity.

At baseline, genetically obese mice are observed to have more Firmicutes than Bacteroidetes compared to their lean counterparts.\textsuperscript{51} In order to support this observation, as well as exclude that this ratio is restricted to genetically obese mice, studies have characterized the gut microbiota of high-fat fed mice. Murphy et al. found an increased Firmicutes and reduced Bacteroidetes proportion in mice fed a high fat diet.\textsuperscript{50} Similar findings were observed in two other high-fat diet mice trials.\textsuperscript{91,92}

In humans, adult female monozygotic and dizygotic twin pairs concordant for leanness or obesity revealed that the obese gut microbiota was associated with significantly lower Bacteroidetes and decreased diversity.\textsuperscript{93} This observation was analyzed in a diet and weight loss study with obese individuals who were assigned to one of two low-
calorie diets: fat or carbohydrate restricted. At baseline, obese people had fewer Bacteroidetes (P<0.001) and more Firmicutes (P=0.002) than the lean controls. After calorie restriction, and over time, Firmicutes decreased significantly (p=0.002) and Bacteroidetes increased significantly (p<0.001) in obese participants. The results showed that irrespective of which two diets were assigned, the lower F:B ratio correlated with weight loss. This study indicates that certain bacteria may be implicated in obesity, and that manipulating the gut communities could be one approach to addressing obesity.

In addition to the observed variation in the F:B ratio due to calorie restriction, diet pattern variations are shown to correlate with changes in the microbiota. For example, a lower ratio of F:B was observed in children from rural Africa consuming a plant-based dietary pattern versus European children consuming a western-style diet. The authors speculated that this change may be a mechanism to maximize energy uptake from their fiber-rich diet. This finding may explain why high fat diets in mice correlated with higher Firmicutes, since the Bacteroidetes phylum specializes in fiber degradation.

While substantial evidence from robust studies support the association between the F:B ratio and obesity, conflicting reports exist in the literature. Schwiertz et al. characterized the fecal microbiota of overweight, obese, and lean adults and found that while the total amount of SCFA was higher in the obese group, consistent with the obesity hypothesis, they found a significantly higher abundance of Bacteroidetes than Firmicutes in overweight and obese subjects compared to lean subjects. In addition, one study found that the F:B ratio did not have a function in determining obesity, at
least at the phylum level, between lean and obese individuals. Further, the obesity associated Western diet has been shown to increase the Bacteroides genus within the Bacteroidetes phylum, but evidence also shows that obese individuals have a higher baseline F:B ratio.

While research has found an association between the gut bacteria composition and obesity, it is difficult to draw conclusions due to conflicting evidence. As such, the link between obesity and the gut microbiota may be more complicated than a shift in the Firmicutes:Bacteroidetes ratio. Therefore, since the link between the microbiota and obesity is inconclusive, it may be beneficial to examine how dietary patterns as a whole impact the gut to determine if an association persists across lifestyle factors.

2.2.5 Diet patterns

2.2.5.1 Western diet

The Western lifestyle is often characterized by high fat and high sugar consumption with a high incidence of chronic diseases including CVD and type II diabetes. Diet and gut health studies have linked the Western diet to unfavorable changes in the gut microbiota. In addition to the effects of high-fat diets already discussed, they can increase microbial production of deoxycholic bile acid (DCA) concentrations, which is a compound associated with liver cancer. Further, DCA was shown to significantly increase Firmicutes while decreasing Bacteroidetes, similar to those observed in mice fed high-fat diets. Diets high in saturated fat have also been found to increase numbers of pro-inflammatory microbes like Bilophila wadsworthia. Additionally, fat in lard form increased toll-like receptor activation and impaired insulin sensitivity versus
consumption of fish oil in mice. The authors concluded that an interaction between gut microbiota and the saturated fats led to these metabolic effects.\textsuperscript{86}

2.2.5.2 Plant-Based

Compared to the western diet, plant-based diets are favored as they tend to produce end products like SCFAs that assist in gut and overall health. Vegetarian and vegan diet studies have substantiated the benefits of plant-based diets. In a pooled analysis of 5 cohort studies, mortality from coronary heart disease (CHD) was reduced 24\% in vegetarians compared with non-vegetarians.\textsuperscript{41} As such, plant-based diets may confer health benefits through modulation of the gut microbiota. A greater abundance of Bacteroidetes with a lower abundance of Firmicutes was observed when consuming a plant-based diet versus consuming a typical western diet.\textsuperscript{87} Conversely, compared to a Western diet, a Japanese diet (rich in soybean, radishes, cabbage, fish, seaweed and green tea) resulted in lower counts of Bacteroides genera and higher counts of Lactobacillus.\textsuperscript{98} Similar findings were observed in those following a vegetarian and vegan diet versus an omnivore control diet–both intervention groups saw significantly lower Bacteroides and Bifidobacterium counts, and vegans had significantly lower E. Coli and Enterobacteriaceae counts.\textsuperscript{99} The discrepancy seen in these plant-based studies may be explained by host genetics, different methodologies, or different microbiome profiling techniques. Nonetheless, this data indicates that different diet patterns, specifically a plant-based pattern, have the capacity to alter the gut microbiota which may or may not be related to positive health outcomes like reduced CHD.
2.2.5.3 Mediterranean

Evidence also reveals that the Mediterranean diet may confer benefits to the host by altering the gut bacteria. As a diet that is plant-based, the Mediterranean diet is encouraged as a healthy eating pattern to establish and maintain good heart health.\textsuperscript{100} Emphasis is placed on consuming high fiber, vegetables, fruit, grains, fish and poultry and minimizing intake of red meat, dairy, and sweets. Further, saturated fat intake should be limited in favor of monounsaturated fatty acids and polyunsaturated fatty acids.\textsuperscript{100} One study found that vegan, vegetarian, and omnivore participants whose diets aligned with the Mediterranean diet had increased fecal SCFAs, Firmicutes, and Prevotella (Bacteroidetes phylum), while low adherence to the diet was associated with elevated TMAO.\textsuperscript{101}

2.2.5.4 Probiotics

Probiotics are live bacteria that, once consumed, benefit the host by colonizing the gut and exerting health promoting functions. Probiotics are prescribed to aid in restoring gut ecology in diseases such as IBS, IBD, enterocolitis, and infectious diarrhea.\textsuperscript{102} Various Lactobacilli and Bifidobacterium strains are recognized as probiotic agents and are thought to restore gut health.\textsuperscript{102} Their mechanisms vary depending on the strain of bacteria and the disease in which it is used to treat, and include maintaining host-microbe interactions and pathogen growth, mucus secretion from goblet cells, maintaining epithelial barrier integrity, and producing antibacterial factors including activation of the host’s adaptive immune system.\textsuperscript{102} In a placebo-controlled randomized controlled trial (RCT), 60 overweight healthy adults consumed probiotics with various
strains of Bifidobacterium, Lactobacilli, and Streptococcus, which resulted in increases in concentration of the same bacteria. Additionally, yogurt with probiotic strains of bacteria reduced counts of enteropathogenic E. coli and Heliobacter pylori in-vitro.

2.2.6 Fruit

Plant-based diets are shown to significantly alter the gut composition, and a large food item consumed in a plant-based diet is fruit. Fruit is currently the second most popular food item in the US, and by sales alone, berries, apples, bananas, grapes, and citrus rank in the top five highest grossing fruits, with berry sales ranking the highest at $3.02 billion. Compared to other berries, strawberry consumption is much greater with an estimated per capita annual consumption of 7.9 pounds per year. With increased accessibility and per capita consumption, high levels of vitamins, minerals, and antioxidants, fruit has the potential to be an effective approach to improving health, specifically through the gut.

Gut health has been associated with the concept of the ‘three P’s which include probiotics, prebiotics, and polyphenols. As such, research has investigated the effect of berries and berry polyphenols on the gut as they have received attention as antioxidants with properties to prevent chronic disease. The gut bacteria convert polyphenols into active and bioavailable metabolites, suggesting that variations in the gut microbiota can affect polyphenol activity and thus, may have short and long-term impacts on human health.
2.2.6.1 Previous work: polyphenols and gut health

While relatively low in polyphenols compared to strawberries, several studies have assessed the potential for non-berry consumer fruits to modulate the gut microbiota. Shinohara et al. found that consumption of two apples per day increased Lactobacillus and Streptococcus while C. perfringens and Enterobacteriaceae decreased.\textsuperscript{13} Oranges and bananas have also been identified as a fruit with the ability to beneficially alter the gut microbiota. In a SHIME (Simulator of the Human Intestinal Microbial Ecosystem) vessel, Duque et. al. found that fresh orange juice significantly increased commensal bacteria species (from genera Lactobacillus, Enterococcus, Bifidobacterium, and Clostridium) while reducing Enterobacteria.\textsuperscript{14} Mitsou et al. assessed the impact of bananas on the gut microbiota and found that 60 days of banana consumption resulted in a non-significant increase in Bifidobacterium levels in the banana group.\textsuperscript{15}

In addition to whole non-berry fruit, two studies have investigated the influence of red wine polyphenols on the human gut. Queipo-Ortuno et al. had 10 healthy men consume 272 ml a day of red wine (797.86 mg gallic acid equivalents [GAE] of total phenols), de-alcoholized red wine (733.02 GAE of total phenols), or gin, each for 20 days.\textsuperscript{9} Red wine polyphenols significantly increased Proteobacteria, Fusobacteria, Firmicutes, and Bacteroidetes, while the de-alcoholized red wine increased Fusobacteria but significantly decreased Firmicutes and Bacteroidetes. The authors concluded that red wine polyphenols exhibit a prebiotic effect. Moreover, changes in cholesterol and C-reactive protein concentrations were linked to changes in Bifidobacterium numbers.\textsuperscript{9} With the same diet supplement, but in participants with MetS, Moreno-Indias et al.
found that red wine and de-alcoholized red wine, consumed for 30 days each, significantly increased the number of Bifidobacterium and Lactobacillus while decreasing Bacteroides, E. coli, and Enterobacter spp. The polyphenols also improved various metabolic markers. The authors concluded that the changes in the MetS participants’ gut microbiota could be responsible for the improvement in the MetS markers.

Polyphenols in the form of fruit extracts can also impact the gut microbiota. Molan et al. assigned thirty healthy men and women to consume blackcurrant extract powder with lactoferrin and lutein or to consume only blackcurrant extract powder in capsule form four times per day for two weeks. Both forms of blackcurrant significantly increased Bifidobacterium and Lactobacilli population sizes while Clostridium spp. and Bacteriodes spp. decreased significantly. The authors concluded that blackcurrant powder can act as a prebiotic. Similarly, in a controlled trial, Li et al. instructed 20 normal weight healthy male and females to consume a daily dose of 1000 mg of pomegranate extract (680 mg GAE of total phenols), equivalent to 8 oz of pomegranate juice, for 4 weeks. Consumption of pomegranate extract significantly increased Actinobacteria with a significant decrease in Firmicutes. The authors proposed that these results may have implications in weight maintenance and insulin resistance by changing the ratio of Firmicutes to Bacteroidetes.

Numerous studies have investigated the effect of whole berries on the composition of the gut microbiota. Specifically, red berries have been analyzed in several controlled diet intervention trials. Vendrame et al. investigated the daily consumption of a wild
blueberry (WB) freeze-dried powder drink in a RCT, crossover, diet intervention.\textsuperscript{16}

Twenty male volunteers with at least one risk factor for CVD consumed a 250 mL WB drink (25g of WB freeze-dried powder; 375 mg anthocyanins) or a placebo drink for 6 weeks. Blueberry polyphenols significantly increased Bifidobacterium spp. after the blueberry treatment with increased Lactobacillus acidophilus after both treatments.\textsuperscript{16}

In another diet intervention, Ige et al. assigned four female volunteers to consume 600 ml of blueberry puree per day for 29 days.\textsuperscript{17} Before stool analysis, the samples were incubated for Lactobacillus spp. and Enterobacteriaceae spp. The authors found that consumption of blueberry puree resulted in new strains of Lactobacillus bacteria while other Lactobacillus strains resisted the anti-oxidant properties of the blueberry.\textsuperscript{17}

In addition to blueberries, raspberries have also been targeted as a fruit rich in polyphenols with the potential to impart health benefits through the gut. In a free-living diet intervention trial, Gill et al. instructed 10 male participants to consume 200 g of raspberry puree (296 mg gallic acid equivalents) per day for 4 days.\textsuperscript{18} Following stool sample analysis, it was observed that the raspberry supplementation resulted in small, yet insignificant changes to the microbiota composition.\textsuperscript{18}

In addition to assessing the impact of single fruits on the gut microbiota composition, one researcher investigated the synergistic effect of a combination of whole red berries on the gut. Puupponen-Pimia et al. assigned 32 male and female participants with MetS to consume either 300 g of fresh berries (70.7 mg anthocyanins) comprised of 100 g strawberry puree, 100 g frozen raspberries, and 100 g frozen cloudberries or to restrict
berry consumption for 8 weeks. Participants maintained their habitual diet but restricted consumption of berries to 80 g/day. Stool samples were collected during five separate laboratory visits and were analyzed for microbial diversity. Results showed that 4 subjects in the berry group saw insignificant changes to their bacterial profile while 13 participants saw no change. Further, no significant differences in diversity of predominant bacterial populations were seen between groups.

Compared to the top fruits consumed in the US, as reviewed in the studies above, berries, including strawberries, contain a wide spectrum of beneficial ingredients, and combined with their affordability and accessibility, give them the potential to improve health through the gut.

2.3 Strawberries

The strawberry (genus: Fragaria) is a member of the Rosaceae family and is widely consumed in the Mediterranean diet due to their diverse nutritional composition. While researchers have just recently begun studying the health benefits of strawberries, the strawberry dates back to the first century A.D. and have been eaten in small quantities by people worldwide since ancient times. It wasn’t until the 1300s when the French transplanted the wild strawberry into the garden that strawberries began to be cultivated and widely consumed. The spread of this berry was slow and was not fully appreciated until the end of the 18th century when the Chilean strawberry was crossed with the Virginia strawberry, giving rise to the modern strawberry known today.
2.3.1 Biochemical composition

The unique biological composition of strawberries yields health benefits through their high content of essential nutrients and beneficial phytochemicals. They exert their effects on human health by impacting lipid profiles, insulin response, immunological responses, and pathogen growth, and thus have implications for heart and gut health.\textsuperscript{109} The nutrients in strawberries that are likely to have the greatest impact on improving human health are fiber, vitamin C (see appendix A for strawberry nutrient composition), and various polyphenols, namely flavanoids, hydrolyzable tannins, and phenolic acids\textsuperscript{109} (Figure 3) (see appendix B for strawberry polyphenol composition). Briefly, as a functional component of strawberries, fiber slows digestion and can control calorie intake through satiation.\textsuperscript{111} Apart from their role in lowering LDL-CH, increasing insulin sensitivity, and aiding in gut motility,\textsuperscript{112} fiber also improves gut health when degraded to SCFAs. Vitamin C, a known antioxidant, participates in gene expression and is a cofactor in enzymatic reactions throughout the body including collagen, carnitine, and neuropeptide synthesis.\textsuperscript{113} Various cohort studies show that vitamin C is associated with lower risk for hypertension, stroke, and coronary heart disease.\textsuperscript{113} The impact of strawberry polyphenols on gut health are specific to each subgroup and will be discussed individually in the following sections.
2.3.2 Effects on heart health

Recent literature has shown that strawberries exhibit beneficial effects on heart health. While the mechanism is still unknown, strawberry polyphenols significantly lowered triglycerides and oxidized LDL-CH (low density lipoprotein-cholesterol) after hyperlipidemic adult men and women consumed a high-fat meal. Additionally, strawberries have been found to significantly decrease total and LDL-CH in adult men and women with MetS while significantly decreasing serum cholesterol levels in overweight and obese men and women. In addition, strawberries appear to exert a protective effect against the development and/or progression of inflammatory conditions such as CVD. For instance, in various LPS treated cell models, including mouse macrophages and human fibroblast cells, strawberries were shown to counteract LPS induced oxidative stress by reducing ROS and nitrite levels; protecting against DNA
damage and lipid and protein oxidation; and reducing pro-inflammatory cytokines (IL-1β and IL-6) while increasing the anti-inflammatory cytokine IL-10.117,118

2.3.3 Effects on gut health

As a rich source of polyphenols, another way strawberries impart their health benefits is through modulation of the gut microbiota. Polyphenols undergo metabolism by the gut microbiota therefore producing metabolites that are more readily available to the body.107 Colonic fermentation of polyphenols yield numerous absorbable biotransformation products including phenylacetic, phenylpropionic, phenylbutiric, valeric acids, valerolactone, and urolithin A and B.119

Once ingested, polyphenols have been shown to promote the growth of bacteria including Lactobacillus and Bifidobacterium.107 In this way, polyphenols exhibit prebiotic like effects and can be viewed as relevant modulators of beneficial microbiota.108 Such effects have been documented in a variety of in-vitro and clinical studies with polyphenol-rich foods, many of which have been detailed in the above text [Section 2.2.2]. Further, recent studies have shown a positive association between consuming polyphenol-rich foods and a lower F:B ratio.90,120,121

2.3.4 Phytochemicals

As previously mentioned, strawberries derive some of their health benefits from nutritive compounds. Strawberries also consist of nonnutritive phytochemical compounds that impart their benefits through the gut. Phytochemicals are plant metabolites that enable the plant to overcome environmental threats while controlling
growth and reproduction. Such effects have prompted researchers to identify phytochemicals with therapeutic potential in humans, including those found in strawberries. The major phytochemicals present in strawberries include anthocyanins, flavonols, flavanols, ellagitannins, and hydroxycinnamic/hydrobenzoic acids.

### 2.3.4.1 Anthocyanins

Anthocyanins represent a major phytochemical group in strawberries with more than 25 different anthocyanin pigments reported including pelargonidins and cyanidins. A meta-analysis by Giampieri et al. details the health benefits of strawberries and explains that anthocyanins avoid absorption in the small intestine and subsequently pass through to the colon where bacteria convert the chemical into smaller phenolic acids. Regarding their impact on human health, one study found that anthocyanins exerted an anti-inflammatory effect in human epithelial cells infected with Helicobacter pylori thereby ameliorated gastric mucosal damage. Another study found that one month of consuming 500 g of strawberries in healthy individuals was associated with improvement of the serum lipid profile. Therefore, the intake of anthocyanin-rich strawberries could potentially prevent gastrointestinal distress and the pathogenesis of CVD.

### 2.3.4.2 Flavonols

Another bioactive compound in strawberries are flavonols which consists of quercetin and kaempferol compounds. Members of the flavonoid class are primarily degraded by Clostridium and Eubacterium. Quercetin has been shown to exhibit prebiotic and anti-microbial potential by stimulating Lactobacillus spp. growth and inhibiting E. coli
while enhancing the intestinal barrier function.\textsuperscript{126} Research also reported the ability of quercetin to attenuate the increase in the F:B ratio in high-fat fed mice.\textsuperscript{90}

2.3.4.3 Flavanols

Flavanols consist of compounds including catechins and proanthocyanidins.\textsuperscript{111} Proanthocyanidins are found in the strawberry flesh\textsuperscript{111} and, like anthocyanins, are processed by the gut bacteria to produce phenolic acids.\textsuperscript{123} In a double-blind crossover RCT, cocoa flavanols increased Bifidobacterium and Lactobacilli populations and decreased Clostridia counts.\textsuperscript{127} Like flavonols, flavanols may also impart health benefits through prebiotic activity.

2.3.4.4 Hydrocinnamic/Hydrobenzoic acids

Hydroxycinnamic hydroxybenzoic acids exist within the phenolic acid group and include caffeic acid, gallic acid, and coumaric acid.\textsuperscript{109} Of the available research, hydrocaffeic acid has been shown to exhibit anti-inflammatory activity in vitro and in vivo, eluding to their anti-cancer properties.\textsuperscript{128}

2.3.4.5 Ellagitannins

Hydrolyzable tannins represent the second major phenolic class in strawberries. Within this class, ellagitannins are the only major group.\textsuperscript{111} Ellagitannins are comprised of various compounds including ellagitannin, ellagic acid, ellagic acid glycosides, sanguin H-6, and galloyl-bis-HHDP-glucose.\textsuperscript{109} Ellagitannins are only found in cloudberry, raspberry, rose hip, sea buckthorn, and strawberry. Notably, strawberries exhibit antimicrobial properties through the activity of ellagitannins.\textsuperscript{109} An in-vitro study found
that phenolic extracts from strawberries elicited antimicrobial activity against B. cereus, H. pylori, C. jejuni, and C. albicans, thereby revealing that ellagitannins are principally involved in pathogen suppression.\textsuperscript{109}

Of specific interest, the gut microbiota can convert ellagic acid into bioavailable urolithins; a class of compounds shown to exhibit anti-inflammatory and anticarcinogenic effects.\textsuperscript{129} The bacteria \textit{Gordonibacter urolithinfaciens} and other unknown species are involved in the conversion of ellagic acid to urolithin A, isourolithin A, and urolithin B.\textsuperscript{107} Urolithins are produced in various concentrations depending on the individual which may have implications for health.\textsuperscript{129} In a study looking at microbial metabolism of ellagic acid, three different urolithin phenotypes were consistently observed in human intervention trials: ‘Phenotype A: produce only urolithin A’, ‘Phenotype B: produce isourolithin A and/or urolithin B in addition to urolithin A’, and ‘Phenotype 0: no detections of urolithins’.\textsuperscript{129} These observations were made independent of age, gender, BMI, health status, or amount and type of ellagitannin food ingested. However, phenotype B was observed in individuals with MetS or colorectal cancer associated with dysbiosis.\textsuperscript{129} Based on these data, the gut microbiota composition may modulate urolithin production and bioavailability, therefore targeting the gut may be beneficial when considering the health benefits of ellagic acid.

\textbf{2.4 Conclusion}

Due to their popularity, accessibility, and high polyphenol content compared to other popular consumer fruits, strawberries have recently emerged as a functional food which
are implicated in disease prevention and health promotion. Specifically, the phenolic compounds found in strawberries stimulate the growth of commensal and probiotic strains of bacteria, and thus, may be an alternative to pharmaceutical interventions for improving gut health. Furthermore, recent research has linked the gut to a variety of cardiometabolic disease states, and thus, diet modification may be one method to promote health through modulating the gut bacteria.

To date, research has not assessed the potential effect of a daily, modest consumption of strawberries (~1 cup/d) on the gut microbiota in overweight, postmenopausal women. Research has shown that CVD risk increases in women with age and the transition through menopause. Since research has shown a significant relationship between diet, the gut microbiota, and risk factors for cardiovascular and metabolic disease, targeted dietary interventions may be effective at improving cardiometabolic health in postmenopausal women through the gut. Therefore, to add to the body of research, and reduce the gap in the literature, the aim of this study is to evaluate the effects of strawberry consumption on gut health in postmenopausal women.
3. MATERIALS AND METHODS

3.1 Objectives

Given the potential role of strawberries on gut health, and the association between gut bacteria and health outcomes, the objectives of this study were to determine the impact of daily strawberry consumption on specific gut health changes in overweight, postmenopausal women. Specifically, we identified if 13 g/d of FDSP would impact the following objectives:

- Objective #1: Firmicutes to Bacteroidetes ratio.
- Objective #2: Bacterial diversity.
- Objective #3: Relative abundance of Lactobacillus and Bifidobacterium.

These objectives were based on the hypothesis that polyphenol intake may modulate the gut microbiota by influencing the growth of specific bacteria linked to host health. Therefore, the hypothesis is that daily consumption of FDSP, equivalent to 1 cup of fresh strawberries, will beneficially affect the composition of the gut microbiota by reducing the ratio of Firmicutes to Bacteroidetes, increasing microbial \( \alpha \)-diversity, and increasing the relative abundance of probiotic Lactobacillus and Bifidobacterium bacteria.

3.2 Participants and Recruitment

Participants were recruited from San Luis Obispo and Fresno counties through flyers, digital, and social media advertisements. Ten weight-stable (≤5% body weight change in previous 6mo), overweight (BMI 25-34.9 kg/m\(^2\)), postmenopausal women (age ~45-70y; >12mo since last menstrual cycle) volunteered to participate in this study (Figure 5).
Participants were generally healthy and had not altered their physical activity levels for at least 6 months prior to the start of the study. Exclusion criteria included:

1. Smoking or tobacco use (current or within the past 6 months);
2. >7 alcoholic beverages/wk OR > 2 servings/day (beverage = 12 oz. beer, 5 oz. wine, 1.5 oz. distilled spirits);
3. Currently following an energy-restricted (intentionally reducing energy intake to lose weight) or low-fat (<20% energy from fat) diet;
4. Regular physical activity level >180min/wk of moderate to high intensity physical activity, excluding activities normally required for participant’s occupation;
5. Planning to begin engaging in moderate to high intensity physical activity >90min/wk after being sedentary for >6 months;
6. Use of medications or supplements that could interfere with the outcomes of this study (including probiotics and antibiotics);
7. Unwilling or unable to consume <5 serv/wk of soy (specifically tofu and soybeans), green tea, or high-cocoa (>60%) dark chocolate, combined;
8. Unwilling or unable to avoid consuming more than 1 serving of red wine per week;

3.3 Screening

Volunteers were screened through a two-step process: 1. Completion of an online or phone eligibility questionnaire and 2. In person verification of BMI. The study website
was hosted on Cal Poly’s Drupal Secure Forms website which included a link to a page with the eligibility screening questionnaire. If the volunteers were eligible based on the screening questionnaire, they were invited to come to the Cal Poly Human Nutrition Lab for an in-person screening to verify BMI. Following the in-person screening, eligible and interested individuals were asked to read and complete the informed consent form and complete a health history questionnaire in order to participate in the study.

3.4 Experimental Design

This study was a 5-week free-living diet intervention trial consisting of a 3-week washout (Figure 4) followed by a 2-week diet intervention treatment.

![Study flow diagram](image)

**Figure 4.** Study flow diagram.

3.5 Intervention

3.5.1 Washout

Once informed consent was obtained, each participant completed a 3-week washout phase which required the participant to avoid consuming dietary sources high in polyphenols and probiotics, including, but not limited to foods or beverages with fresh, frozen, or processed berries. Participants were also asked to consume ≤5 servings per
week of tofu, soybeans, green tea, and high-cocoa chocolate. A list of foods to avoid was given to participants to reference for the duration of the study (see appendix C).

3.5.2 Health Check #1 and #2

After the participants successfully completed the washout, they proceeded to the first Health Check. “Health Check #1” was a baseline set of assessments including anthropometrics, two fecal collections, and 3-day diet and physical activity records. After the washout, participants began the intervention and then ended the study with “Health Check #2”. The same health check assessments that were performed in Health Check #1 were performed during Health Check #2.

3.5.3 Supplement intervention

For 2-weeks during the supplement intervention, participants consumed 13 g/d of FDSP, equivalent to ~125 g fresh strawberries (~1 cup fresh), in 4-8 oz of water every day. To ensure palatability, participants were given the option to mix into a smoothie form with banana, orange juice, or ice (one participant mixed strawberry powder with almond milk). The supplement composition is as follows:

Freeze-dried strawberry powder – 13 g FDSP; composed of dehydrated strawberries representing a mixture of cultivars commonly available to consumers in the United States as fresh and frozen berries (See Appendix A and B for nutrient composition and polyphenol composition of FDSP).
3.6 Assessment Procedures

A brief schedule of assessments completed by the participants is shown below (Table 1).

A week-by-week breakdown of the study activities was given to participants along with a calendar that depicted when to collect the stool samples and when to complete the 3-day weighted diet and physical activity records.

Table 1. Assessments.

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>Assessment Time Point (Wk)</th>
<th>Assessments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>0</td>
<td>Anthropometrics, Health History Questionnaire</td>
</tr>
<tr>
<td>Washout</td>
<td>3</td>
<td>Anthropometrics, Gut Microbiota, Diet &amp; PA</td>
</tr>
<tr>
<td>Supplement Intervention</td>
<td>5</td>
<td>Anthropometrics, Gut Microbiota, Diet &amp; PA</td>
</tr>
</tbody>
</table>

*PA=Physical activity

This study was approved by Cal Poly Institutional Review Board (IRB#2018-277-CP).

3.6.1 Anthropometrics

Both body weight and height were assessed at baseline. Weight was determined using a Seca scale (seca 876, Seca GmbH & Co. KG, Hamburg, Germany). Height was determined using a Seca stadiometer (seca 217, Seca GmbH & Co. KG, Hamburg, Germany). Body weight was assessed after the 3-week washout and following the 2-week intervention period. BMI was calculated from weight and height.

3.6.2 Diet, Physical Activity, & Health History

Diet, PA, and health history was assessed at baseline using a Health History Questionnaire. Three-day weighed food and PA records (see Appendix D and E) were
used to document, track, and assess dietary intake and PA and were performed within the week before each health check. Participants used a tracking log to document berries and other foods high in probiotic and polyphenolic that may have been consumed throughout the study. Diet and PA data were analyzed using ESHA Food Processor (v10.14.2).

3.6.3 Gut Microbiota Health - Fecal Analysis

Following the washout and supplement intervention, participants provided 2 fecal samples over a 4-7 day period during the last week of the washout phase and within the two days following the end of the supplement intervention. Participants were provided with uBiome’s stool Gut Explorer™ collection kit (Figure 5) that included instructions and return procedures along with all necessary supplies. All participants were given gloves to reduce the risk of fecal contamination. The kit is equipped with the following materials: a collection vial, swabs, a replacement vial, a sample return bag in which the sample is placed before putting in the return mailer, and a return mailer (with prepaid postage by uBiome). All participants collected their stool samples according to protocol outlined in the kit. Briefly, following a bowel movement, participants were instructed to use a sterile swab to transfer a small amount of fecal material into a vial containing a proprietary lysis and stabilization buffer that preserves the DNA for transport to the uBiome research facility at ambient temperatures. Participants obtained the stool sample by wiping the swab to the soiled toilet paper and then swirled the swab in the vial for one minute. After collection, participants mailed their de-identified samples to the uBiome research facility for DNA extraction and 16S rRNA gene sequencing.
3.6.4 DNA extraction and 16S rRNA Gene Sequencing

According to uBiome protocol, samples were lysed using bead-beating, and microbial DNA was extracted in a class 1000 clean room by a guanidine thiocyanate silica column-based approach using a liquid-handling robot. Polymerase chain reaction (PCR) amplification of the 16S rRNA genes was performed using universal V4 primers (515F: GTGCCAGCMGCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT). Samples were barcoded with a unique combination of forward and reverse indexes allowing for simultaneous processing of multiple samples. PCR products were pooled, column-purified, and size-selected through microfluidic DNA fractionation. Consolidated libraries were quantified by quantitative real-time PCR using the Kapa Bio-Rad iCycler qPCR kit on a BioRad MyiQ before loading into the sequencer. Sequencing was
performed in a pair-end modality on the Illumina NextSeq 500 platform rendering 2 x 150 bp pair-end sequences.\textsuperscript{130}

\section*{3.7 Compensation}

Upon completion of all study requirements, participants were compensated with a $50 gift card to Target or Amazon based on their preference.
4. RESULTS

4.1 Participants

A total of 31 participants completed the online screening questionnaire, 12 of whom qualified for the study based on the questionnaire (Figure 6). At California Polytechnic State University-SLO, 5 participants were screened to confirm their final eligibility, and each qualified. At the Eye Medical Center of Fresno, 7 participants were screened to confirm their final eligibility, and each qualified. Of the 12 total participants who qualified, 10 completed the study: 4 from San Luis Obispo (SLO) and 6 from Fresno. Reasons for participant drop-out included health concerns and stomach discomfort from the supplement.
Figure 6. Consort flow diagram of participant participation.
Baseline characteristics are shown in Table 2. None of the characteristics were significantly different between participants from SLO or Fresno.

Table 2. Participant baseline characteristics, mean ± SD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SLO (n=4)</th>
<th>Fresno (n=6)</th>
<th>Mean (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59 ± 6.27</td>
<td>61.5 ± 11.11</td>
<td>60.5 ± 9.13</td>
<td>0.70</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>75.9 ± 9.98</td>
<td>73.91 ± 11.88</td>
<td>74.71 ± 10.61</td>
<td>0.79</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.7 ± 4.05</td>
<td>28.83 ± 3.03</td>
<td>29.98 ± 3.58</td>
<td>0.23</td>
</tr>
</tbody>
</table>

SLO=San Luis Obispo  
BMI=Body Mass Index

4.2 Measures of Adiposity

Results for body weight and BMI among all 10 participants are shown in Table 3.

Table 3. Measures of adiposity at baseline, week 3, and week 5, (n=10).

<table>
<thead>
<tr>
<th>Measure of Adipositya</th>
<th>Baselineb</th>
<th>Week 3b</th>
<th>Week 5b</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>74.71 (3.30)</td>
<td>74.15 (3.30)</td>
<td>74.39 (3.30)</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.98 (1.11)</td>
<td>29.76 (1.11)</td>
<td>29.80 (1.11)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

aParticipants were categorized as a random effect  
bLeast squares mean (standard error of the mean)  
BMI=Body Mass Index

There were no significant differences in body weight between baseline and subsequent weeks. Average body weight at baseline was 74.71 kg and was 74.15 kg and 74.39 kg on week 3 and week 5 respectively.

Similarly, there were no significant differences in BMI between baseline and subsequent health checks. Average BMI at baseline was 29.98 kg/m² and was 29.76 kg/m² and 29.80 kg/m² at the end of week 3 and week 5 respectively.
4.3 3-Day Diet Record

Results from the 3-Day Diet records including calorie count and macronutrient and micronutrient intake are show in Tables 4-7 below.

**Table 4. Calorie count and macronutrient intake, (n=10).**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Week 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Week 5&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1634.39 (148.82)</td>
<td>1618.02 (145.65)</td>
<td>0.78</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>202.83 (20.54)</td>
<td>205.31 (15.40)</td>
<td>0.84</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>57.48 (5.38)</td>
<td>60.37 (7.38)</td>
<td>0.48</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>68.34 (6.39)</td>
<td>63.11 (8.23)</td>
<td>0.28</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>20.92 (2.16)</td>
<td>19.14 (1.93)</td>
<td>0.34</td>
</tr>
<tr>
<td>Fiber (per 1000 kcal)</td>
<td>13.01 (0.93)</td>
<td>12.11 (1.12)</td>
<td>0.54</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>66.02 (6.72)</td>
<td>80.49 (7.51)</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Least squares mean (standard error of the mean)

<sup>b</sup>Significant value (p≤0.05)

CHO=Carbohydrate

There were no significant differences in calories, carbohydrates, protein, fat, or fiber between week 3 and week 5. There was a significant difference in sugar intake between week 3 and week 5 (p=0.03). Sugar intake at the end of week 3 was 66.02 (6.72) g while at the end of week 5 increased to 80.49 (7.51) g. Sugar intake was not significant (p=0.27) when the sugar from the FDSP (7.96 g) was removed from the analysis.
Figure 7. Participant calorie, CHO, protein, and fat intake at week 3 and week 5.
Figure 8. Participant fiber and sugar intake at week 3 and week 5.
The calorie count and macronutrient intake varies between participants from week 3 to week 5 (Figure 7 and 8).

**Table 5.** Vitamin intake, (n=10).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Week 3</th>
<th>Week 5</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-RAE (μg)</td>
<td>464.49 (66.61)</td>
<td>471.76 (65.38)</td>
<td>0.94</td>
</tr>
<tr>
<td>D (μg)</td>
<td>1.91 (0.38)</td>
<td>2.45 (0.45)</td>
<td>0.19</td>
</tr>
<tr>
<td>E (mg)</td>
<td>8.01 (2.55)</td>
<td>6.39 (0.99)</td>
<td>0.40</td>
</tr>
<tr>
<td>K (μg)</td>
<td>85.38 (22.95)</td>
<td>47.84 (14.19)</td>
<td>0.20</td>
</tr>
<tr>
<td>B1 (mg)</td>
<td>0.97 (0.22)</td>
<td>0.92 (0.20)</td>
<td>0.43</td>
</tr>
<tr>
<td>B2 (mg)</td>
<td>1.26 (0.18)</td>
<td>1.33 (0.20)</td>
<td>0.46</td>
</tr>
<tr>
<td>B3 (mg)</td>
<td>12.02 (1.77)</td>
<td>13.70 (2.66)</td>
<td>0.33</td>
</tr>
<tr>
<td>B5 (mg)</td>
<td>3.11 (0.34)</td>
<td>2.70 (0.44)</td>
<td>0.39</td>
</tr>
<tr>
<td>B6 (mg)</td>
<td>1.07 (0.16)</td>
<td>1.19 (0.20)</td>
<td>0.33</td>
</tr>
<tr>
<td>Biotin (B7) (μg)</td>
<td>12.16 (3.68)</td>
<td>7.76 (2.54)</td>
<td>0.13</td>
</tr>
<tr>
<td>Folate (B9) (μg)</td>
<td>300.13 (55.16)</td>
<td>263.73 (38.73)</td>
<td>0.36</td>
</tr>
<tr>
<td>B12 (μg)</td>
<td>1.79 (0.30)</td>
<td>3.04 (0.56)</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C (mg)</td>
<td>84.83 (18.23)</td>
<td>106.22 (11.39)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<sup>a</sup>Least squares mean (standard error of the mean)
<sup>b</sup>Significant value (p≤0.05)

There were no significant differences in any vitamin except for vitamin B12 (p=0.03).

The average vitamin B12 intake at the end of week 3 was 1.79 (0.30) μg and was 3.04 (0.56) μg at the end of week 5.
Table 6. Mineral intake, (n=10).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Week 3a</th>
<th>Week 5a</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>625.02 (93.66)</td>
<td>641.46 (104.92)</td>
<td>0.72</td>
</tr>
<tr>
<td>Chromium (μg)</td>
<td>0.59 (0.21)</td>
<td>1.04 (0.37)</td>
<td>0.12</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>0.91 (0.22)</td>
<td>0.63 (0.11)</td>
<td>0.08</td>
</tr>
<tr>
<td>Fluoride (mg)</td>
<td>1.52 (0.56)</td>
<td>1.02 (0.29)</td>
<td>0.46</td>
</tr>
<tr>
<td>Iodine (mcg)</td>
<td>38.33 (8.66)</td>
<td>34.25 (9.94)</td>
<td>0.35</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>12.30 (3.03)</td>
<td>12.61 (3.01)</td>
<td>0.71</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>226.71 (47.02)</td>
<td>180.45 (26.18)</td>
<td>0.19</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>2.25 (0.95)</td>
<td>1.33 (0.27)</td>
<td>0.27</td>
</tr>
<tr>
<td>Molybdenum (mg)</td>
<td>15.36 (6.44)</td>
<td>7.67 (2.84)</td>
<td>0.11</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>805.94 (157.82)</td>
<td>734.79 (123.41)</td>
<td>0.51</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1772.9 (163.31)</td>
<td>1696.42 (148.07)</td>
<td>0.69</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>50.89 (9.23)</td>
<td>45.08 (6.37)</td>
<td>0.47</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2165.8 (271.40)</td>
<td>2193.65 (274.56)</td>
<td>0.89</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>6.13 (1.22)</td>
<td>6.28 (1.04)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

aLeast squares mean (standard error of the mean)

There were no significant differences in mineral intake between week 3 and week 5.

Results for fruit and vegetable intake are shown in Table 7 below.

Table 7. Fruit and vegetable intake, (n=10).

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Week 3a</th>
<th>Week 5a</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit (cup equivalent)</td>
<td>0.87 (0.21)</td>
<td>2.09 (0.28)</td>
<td>0.0014b</td>
</tr>
<tr>
<td>Vegetable (cup equivalent)</td>
<td>1.56 (0.32)</td>
<td>1.22 (0.27)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

aLeast squares mean (standard error of the mean)
bSignificant value (p≤0.05)

There was a significant difference in fruit intake (p=0.0014) between week 3 and week 5. Fruit intake was not significant (p=0.24) when the 1 serving of fruit from the FDSP was removed from the analysis. There was no significant difference in vegetable intake between week 3 and week 5.
4.4 **Gut Health**

Strawberry consumption did not result in a significant change to the F:B ratio or $\alpha$-diversity. Likewise, Bifidobacterium and Lactobacillus abundance did not significantly change.

4.4.1 **Bacteria abundance and alpha diversity**

Table 8 and the following graphs (Figure 8-10) depict changes in Firmicutes, Bacteroidetes, Bifidobacterium, Lactobacillus, and $\alpha$-diversity between week 3 and week 5.

**Table 8.** Bacteria abundance and alpha diversity, (n=10).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Week 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Week 5&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>49.67% (2.72)</td>
<td>51.58% (3.03)</td>
<td>0.97</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>35.18% (2.11)</td>
<td>38.34% (2.45)</td>
<td>0.09</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>0.50% (0.19)</td>
<td>0.68% (0.27)</td>
<td>0.24</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.16% (0.13)</td>
<td>0.47% (0.45)</td>
<td>0.18</td>
</tr>
<tr>
<td>$\alpha$-Diversity</td>
<td>1.83% (0.07)</td>
<td>1.78% (0.09)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

<sup>a</sup>Least squares mean (standard error of the mean)
Figure 9. Abundance of Firmicutes and Bacteroidetes at week 3 and week 5.

There was a non-significant increase in Firmicutes and Bacteroidetes between week 3 and week 5.
Figure 10. Abundance of Bifidobacterium and Lactobacillus at week 3 and week 5.

There was a non-significant increase in Bifidobacterium and Lactobacillus between week 3 and week 5.
Four.5 Statistical methods

Statistical analysis was performed on a personal computer with JMP software (SAS Institute Inc., Cary, NC). Analysis of variance was used to evaluate variance in both body weight and BMI between baseline and the two health checks. Paired t-test was used to evaluate any variance in calories, macronutrients, vitamins, and minerals consumed by the participants between week 3 and week 5. Paired t-test was used to evaluate $\alpha$-diversity and relative abundance of the different bacterial groups between week 3 and week 5. In all statistical tests performed, P values of ≤0.05 were considered significant. Analysis of anthropometrics, 3-day diet records, and bacterial abundance and diversity were performed on 10 participants.
5. DISCUSSION

The present study assessed the effect of strawberries (13 g FDSP/d) on gut health in a 5-week diet intervention trial with one treatment group. Strawberry consumption was not associated with significant changes in measures of adiposity (i.e. body weight, BMI) or changes in diet (i.e. macronutrients, vitamins, minerals) except for sugar, vitamin B12, and fruit consumption. There were no significant differences in $\alpha$-diversity or any bacteria phyla or genera. The data suggests that strawberry consumption at 13 g/d FDSP does not result in significant changes to obesity-associated gut microbiota or beneficial bacteria genera.

The current research is unique compared to previous research for several reasons. The population was composed of postmenopausal women, a population that is strikingly underrepresented in nutrition research. Postmenopausal women were selected as the study population as they are a target for gut dysbiosis and exhibit increased CVD risk. As previously discussed, studies show that polyphenol rich fruits like strawberries may ameliorate dysbiosis and decrease CVD risk. In addition, to our knowledge, three studies\textsuperscript{131-133} have investigated diet and gut health in postmenopausal women, while no study has assessed the effect of strawberries and gut health in postmenopausal women. One strawberry diet intervention study analyzed the combined effect of strawberry puree, raspberries, and cloudberries on gut health,\textsuperscript{19} however, the population was not comprised of postmenopausal women and the intervention material was not strictly
strawberries. Furthermore, this is the first study to evaluate the effect of strawberries on gut health in postmenopausal women.

5.1 Measures of Adiposity

Participants did not exhibit significant differences in any measure of adiposity from baseline to week 5, nor were any significant differences seen between population groups. While no previous research has evaluated the effect of strawberry consumption on body weight or BMI in postmenopausal women, these findings are consistent with other gut health diet interventions. In a 6-wk RCT evaluating probiotic strains and fiber on gut composition in overweight postmenopausal women, there were no significant differences in anthropometric measures compared with placebo.\textsuperscript{132} Similarly, in a red wine polyphenol gut health study, researchers did not observe a significant difference in weight from baseline to end of intervention.\textsuperscript{9} Likewise, a 4-wk RCT assessing cocoa-derived flavanols on gut health found no significant change in BMI.\textsuperscript{127} Weight loss or gain was not anticipated for the current research and the data reflects this.

5.2 3-Day Diet Records

This study was supplementary in nature and therefore did not include a controlled diet regimen; however, in the course of 5-weeks, participants did not show any significant differences in macronutrients, vitamins, or minerals except for sugar (g), vitamin B12 (\(\mu g\)), and fruit consumption. Fruit consumption increased, but upon further analysis, the 1 serv/d fruit from the FDSP accounted for the significant increase in fruit consumption. Therefore, it may be the case that any changes in the gut microbiota, while statistically
insignificant, were a response to strawberry supplementation. Likewise, the relatively high sugar content of 13 g FDSP explained the significant increase in sugar intake between week 3 and week 5. This reveals that strawberry intake significantly contributed to overall sugar consumption.

5.3 Gut Microbiota

Following strawberry supplementation, participants did not show a significant change in bacteria abundance (i.e. Firmicutes, Bacteroidetes, Bifidobacterium, Lactobacillus) or α-diversity. Our hypothesis was that we would see a decrease in Firmicutes and/or an increase in Bacteroidetes (leading to a decrease in F:B ratio), an increase in Bifidobacterium and Lactobacillus, and that there would be an increase in α-diversity following diet intervention. However, at the end of the study, there was no statistically significant change in any of these three objectives.

Previous research of similar study design and duration also did not detect significant changes in the relative abundance of gut microbiota as demonstrated by raspberry puree supplementation\(^\text{18}\) and supplementation consisting of a mixture of berries (including strawberries).\(^\text{19}\) Conversely, a range of high polyphenol fruits in both mice and human models appear to have the ability to significantly alter the gut microbiota composition in ways that may benefit the host.\(^\text{9,11,16,134}\) Notably, strawberry powder supplementation (~167 g fresh strawberry) for 37 days increased α-diversity and alleviated dysbiosis by increasing probiotic bacteria (e.g. Bifidobacterium and Lactobacillus) in mice with colitis. Additionally, strawberry powder (~160 g fresh
strawberry) administered for 10 weeks was shown to increase Bifidobacterium in diabetic mice. However, 2-weeks of strawberry consumption at the current dose (~125 g fresh strawberry) did not alter the microbiota in postmenopausal women in a statistically significant way.

The finding that there was no significant change in bacteria and diversity could be due to sample size, supplement dosing, study duration, and interindividual variability in bacteria composition. The study’s small sample size resulted in less accurate mean values, and therefore less power to detect change. The lack of change possibly indicates that the supplement dose was not high enough to detect significant change or that the study duration was not sufficiently long. It is possible that a higher dose of strawberry or a longer duration may encourage growth of different strains of bacteria that may have been reflected in significant values and an increased alpha diversity. Research indicates that freeze dried blueberries at a dose of 25 g (375 mg anthocyanins) for six weeks results in significant changes in the gut composition. Considering that blueberries have a much higher polyphenol concentration than strawberries, a dose of strawberries at least this amount (while adjusting for density) should be implemented in future studies. Additionally, the variability in gut composition between participants renders it difficult to measure the true efficacy of the intervention. While the increase in Bifidobacterium and Lactobacillus was insignificant, participants eliminated polyphenol foods from their diet, so in theory, the slight increase in these beneficial bacterial could have resulted from the strawberry. These preliminary findings, while valuable to shape future research methods, cannot be generalized to the current target population.
While no significant shifts in the F:B ratio were measured, it is important to note that we were not able to measure total bacteria count. It is known that bacteria such as Firmicutes and Bacteroidetes ferment plant-based substances and thus it is likely that introducing FDSP into the diet increased the total bacteria load. Bacterial load can vary greatly in response to factors such as diet and health; a recent study published in Nature found that healthy individuals compared to unhealthy individuals have one order of magnitude higher bacteria load. This indicates that given a longer length of an increased polyphenol diet, a significant effect on the gut microbiota may have occurred—at some point, there may have been enough growth of total bacteria populations to change the ratio significantly.

With this consideration, the association between a higher F:B ratio and obesity may be a spurious conclusion, since it does not disclose information about total bacteria populations or changes in these populations. The ratio only reveals that the relative population growth or decline causes one phylum of bacteria to grow or decline to shift the ratio, but there is no way to know, for example, the rate at which Firmicutes is changing compared to Bacteroidetes. It could be that both phyla increased but one just increased faster than the other, resulting in a shift in the ratio.

Theory says that obese individuals have a high baseline F:B ratio, but considering the current data, since all we could test for is ratio, an alternative consideration may be that the unhealthy gut conditions associated with obesity simply lowers overall populations of bacteria. It may also be that diets that generally lead to obesity may be less favorable to large populations of gut bacteria as opposed to plant-based diets that encourage
fermentation and therefore larger populations of bacteria. Total bacteria population numbers may be a better indicator of gut health than ratio, which may explain why discrepancies regarding the F:B ratio exist in the literature. Recent literature supports the observation that there is currently no taxonomic signature of obesity that exists in the gut microbiome. Furthermore, the F:B ratio has been shown to both negatively and positively correlate with BMI, so perhaps limiting the study population by this measure is premature. It may be beneficial to determine at what dose strawberry polyphenols affect the gut microbiota in postmenopausal women before restricting the population by weight.

5.4 Conclusion

In the context of gut health, studies have emphasized the importance of analyzing whole diets versus evaluating changes in microbial populations from isolated compounds. Foods contain different mixtures of fiber and polyphenols, and results from studies analyzing single compounds could reach different conclusions from conclusions drawn from the context of real life. As such, the strawberries may work synergistically via its numerous health promoting compounds to confer health to the host through the gut. For this reason, this study analyzed how whole strawberry consumption in a diet may favorable alter the gut microbiota.

In addition, while there is a wealth of literature supporting the association between ratios of bacteria phyla and obesity, the pathogenesis of obesity is multifactorial and is likely more complicated than a simple shift in Firmicutes and Bacteroidetes. Much of the
supporting evidence linking the F:B ratio to obesity was from murine studies which complicates the extrapolation of mouse research to humans. It could be more beneficial to first look at the absolute quantity of microbes and evaluate how these numbers correlate with disease and subsequently see how different species and their metabolites influence health.

5.5 **Strengths and Limitations**

This study was short in duration to minimize attrition and maximize participation. Another strength is that the free-living nature of the study closely mimics reality which allows the results to be better generalized to other populations. Additionally, participants were given individually labeled, pre-packaged, single serving strawberry powder for each day of the week. This enabled participants to easily keep track of their intake, therefore ensuring they consumed the proper quantity of strawberry each day. Another strength is that the portion size and type of strawberries (fresh and frozen cultivars available throughout the US) as well as the concentration and type of polyphenols was likely consistent within each individual and between subjects since participants were consuming homogenized powder from the same batch. Further, uBiome was utilized for all stool sample processing making it possible to expand the study to Fresno county. Samples were shipped directly to the lab, as opposed to processing at Cal Poly, giving the study a high degree of flexibility. Outsourcing sample analysis also decreased chances of sample contamination by human error and minimized human exposure to potentially harmful pathogens. Lastly, a washout period
was utilized to standardize the detection of the effect of the strawberry powder by reducing inter-subject variation.

This study is limited by the relatively small sample size – a larger sample size may have yielded more power to detect significant results. However, pilot studies do not have a defined sample size. Additionally, no control group was used so we cannot conclude for certain the true effect of the treatment. A cross-over RCT would be the preferred study design for a diet intervention of this type. Additionally, a more restricted diet as opposed to free-living may have better standardized detection of changes by reducing intersubject variability. Finally, the sample of feces collected was very small and may not have been representative of the overall proportion of feces to bacteria that inhabit the intestine.

5.6 Future Research

As this is a pilot study, it was designed as a preliminary study for a larger 18-week study being conducted by future graduate students in the department of Food Science and Nutrition at Cal Poly-SLO. The 18-week study will look at the effect of strawberries on gut health in addition to their impact on heart health. The methodology tested in this research will have established a more seamless process to recruit and screen participants, was effective in troubleshooting any equipment problems, and familiarized the study team with procedures. Furthermore, a pilot study enabled the main study to run more smoothly by solving many of the minor problems encountered during the pilot phase.
In addition, the data generated gave a first impression of the variability of the data and established feasibility given the population. This study provided the opportunity to evaluate the representativeness of the sample in addition to the relative cost and time necessary to conduct it. Even with the small sample size, the data can still be used to draw basic conclusions to help inform the 18-week study. Since we used resources to minimize labor and cost, the 18-week study with better reflect any potential effect the strawberries may have on heart and gut health.
REFERENCES


103. Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. *Mediators Inflamm.* 2014;2014:348959.


## APPENDICES

### A. Nutrient composition of freeze-dried strawberry powder*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximates</strong></td>
<td></td>
</tr>
<tr>
<td>Water (g)</td>
<td>10.3</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>4.31</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>350</td>
</tr>
<tr>
<td>Calories from Fat (kcal)</td>
<td>15.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>6.83</td>
</tr>
<tr>
<td>Total lipid (g)</td>
<td>1.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>76.9</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>14.3</td>
</tr>
<tr>
<td>Sugars, total (g)</td>
<td>61.2</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>28.8</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>24.4</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>8.1</td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium (ppm)</td>
<td>1730</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>32.1</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>16800</td>
</tr>
<tr>
<td>Sodium (ppm)</td>
<td>102</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>346</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.06</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.048</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.4</td>
</tr>
<tr>
<td>Pantothenic acid (mg)</td>
<td>0.115</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.0207</td>
</tr>
<tr>
<td>Folate (mg)</td>
<td>0.0399</td>
</tr>
</tbody>
</table>

* Source: California Strawberry Commission
B. Polyphenol composition of freeze-dried strawberry powder. Estimated values*

<table>
<thead>
<tr>
<th></th>
<th>Per 13 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics (mg)$^1$</td>
<td>521.58</td>
</tr>
<tr>
<td>Total anthocyanins (mg)$^2$</td>
<td>40.04</td>
</tr>
<tr>
<td>Ellagic acid (mg)</td>
<td>10.66</td>
</tr>
</tbody>
</table>

*Adapted from Basu, 2009$^{138}$

$^1$expressed as mg gallic acid equivalents  
$^2$expressed as mg cyanidin-3-glucoside equivalents
C. List of food sources high in probiotics and polyphenols

*Strawberries and Gut Health in Post-menopausal Women*

Dietary Intake Directions for Washout & Supplement Intervention Periods

While participating in this study, please maintain your current diet pattern, but avoid dietary sources that are high in polyphenols and probiotics. In addition, do not consume greater than 5 servings a week of soy, green tea, or cocoa products. In addition to avoiding the foods listed below, please adhere to the following directions during your participation in this study:

- Do not smoke or consume tobacco
- One alcoholic beverage is equal to 12 oz beer, 5 oz wine, and 1.5 oz distilled spirit
- Do not follow an energy restricted or low-fat diet (<20% energy from fat)

<table>
<thead>
<tr>
<th>Probiotic Foods</th>
<th>Avoid</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt (limit to ½ cup or 4 ounces per day)</td>
<td>Yogurts that do not contain 'Live &amp; Active Cultures' seal</td>
<td>Kefir</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoid</td>
</tr>
<tr>
<td>Kombucha tea</td>
<td></td>
<td>Avoid</td>
</tr>
<tr>
<td>Tempeh</td>
<td></td>
<td>Avoid</td>
</tr>
<tr>
<td>Cultured Condiments (horseradish, pickle relish, sauerkraut)</td>
<td>Avoid</td>
<td></td>
</tr>
<tr>
<td>Natto</td>
<td></td>
<td>Avoid</td>
</tr>
<tr>
<td>Probiotic and Prebiotic Supplements</td>
<td></td>
<td>Avoid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polyphenol Sources (other than what is provided during diet intervention)</th>
<th>AVOID</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol - wine &amp; beer (limit to &lt;5 servings per week)</td>
<td></td>
<td>Apple juice, Pineapple Juice, Grape Juice</td>
</tr>
<tr>
<td>Green Tea, Black Tea, Oolong Tea</td>
<td></td>
<td>Chamomile tea</td>
</tr>
<tr>
<td>Cocoa Powder and associated products: high-cocoa, polyphenol-rich chocolate (i.e. semi-sweet chocolate; dark chocolate &gt; 60% chocolate)</td>
<td></td>
<td>White Chocolate</td>
</tr>
<tr>
<td>Berries (strawberries, blueberries, blackberries, cranberries, raspberries, etc.) (limit to ≤ 1 cup/wk)</td>
<td></td>
<td>Melons (cantaloupe, honeydew, watermelon), Kiwi (green), Mango, Pineapple, Apples, Figs, Canned Peaches</td>
</tr>
<tr>
<td>Plums</td>
<td></td>
<td>Persimmons</td>
</tr>
<tr>
<td>Grapes (red &amp; black)</td>
<td></td>
<td>Grapes (green), Dried Cranberries, Raisins</td>
</tr>
<tr>
<td>Pomegranates (≤1/wk)</td>
<td></td>
<td>Bananas</td>
</tr>
<tr>
<td>Cherries</td>
<td></td>
<td>Fruit jellies &amp; jams</td>
</tr>
<tr>
<td>Nuts (pecans, hazelnut, walnut) *OK to consume if already part of daily diet</td>
<td></td>
<td>Almonds, Cashews, Macadamia nuts, Peanuts, Pistachios</td>
</tr>
</tbody>
</table>
### D. Example template of 3-Day Food Record

**Strawberries and Gut Health in Postmenopausal Women**  
**3-Day Diet Record**

At the end of each washout and diet intervention period, use this worksheet to record (in detail) all foods and beverages you consume throughout the day.

**Instructions to Remember:**
1. **Start a new page for each day of recording.** Note: the exact days that you record your diet intake should correspond to the exact days you record your physical activity on the 3-Day Physical Activity record forms.
2. Maintain your current eating patterns. Any foods recorded on this form should represent your usual intake.
3. List any vitamin, mineral, or other supplements taken on the back side of this worksheet.

* Do not forget to include water and alcohol in this record.

*Examples are shown in the shaded rows.

<table>
<thead>
<tr>
<th>Time</th>
<th>Meal/Snack</th>
<th>Food or Beverage Item (Name and Description)</th>
<th>Brand/Source (manufacturer, if available)</th>
<th>Preparation Method (bake, boil, fry, etc.)</th>
<th>Amount/Wt (ounces, grams, fluid ounces, cups, tsp, Tbsp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:15 am</td>
<td>Total Cereal</td>
<td>General Mills</td>
<td>NA</td>
<td></td>
<td>1 oz/1 cup</td>
</tr>
<tr>
<td>8:15 am</td>
<td>Light Soy milk, vanilla</td>
<td>Silk</td>
<td>NA</td>
<td></td>
<td>4 fl oz/.5 cup</td>
</tr>
</tbody>
</table>
E. Example template of 3-Day Physical Activity Record

*Strawberries and Gut Health in Postmenopausal Women*

**3-Day Physical Activity Record**

| Study ID: ____________________ | Date: ________________ |

Use this worksheet to record any physical activity you engage in during the last week of the washout and/or diet intervention study phases (depending on which study phase you are in).

**Instructions:**
1. Maintain your current level of physical activity. Any activity recorded on this form should represent your usual level/intensity of exercise.
2. Start a new page for each day of recording.
3. Activity record should correspond to the exact days of the 3-Day Diet Record.
4. Please refer to the definitions of low, moderate, and high intensity (shown below) when completing your records.

* 

<table>
<thead>
<tr>
<th>Low Activity</th>
<th>Moderate Activity</th>
<th>High Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>requires minimal to no effort with no change in heart rate</td>
<td>requires a moderate amount of effort and causes increased breathing with a moderate increase in heart rate</td>
<td>requires a large amount of effort and causes rapid breathing and a substantial increase in heart rate</td>
</tr>
<tr>
<td>□ walking slowly</td>
<td>□ walking briskly</td>
<td>□ jogging/running</td>
</tr>
<tr>
<td>□ sitting at computer</td>
<td>□ heavy cleaning (washing windows, vacuuming, mopping)</td>
<td>□ mountain climbing</td>
</tr>
<tr>
<td>□ standing light work (cooking, washing dishes)</td>
<td>□ mowing lawn</td>
<td>□ bicycling more than 10mph</td>
</tr>
<tr>
<td>□ stretching</td>
<td>□ bicycling lightly</td>
<td>□ step aerobics</td>
</tr>
<tr>
<td>□ fishing</td>
<td>□ hiking</td>
<td>□ jump roping</td>
</tr>
<tr>
<td>□ playing catch</td>
<td>□ recreational swimming</td>
<td>□ treading water</td>
</tr>
</tbody>
</table>

**Strawberries and Gut Health in Postmenopausal Women**

**3-Day Physical Activity Record**

| Study ID: ____________________ | Date: ________________ |

Examples are shown in the shaded rows.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Physical Activity performed</th>
<th>Description of Activity</th>
<th>Intensity (*low, mod., high)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00am</td>
<td>Jogging</td>
<td>Jogged around neighborhood</td>
<td>Moderate</td>
<td>15 minutes</td>
</tr>
</tbody>
</table>

| | | | | |