IN VITRO FERMENTATION BY GUT MICROBIOTA OF HUMAN SUBJECTS FROM THREE ETHNIC GROUPS IN HAWAII

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ABSTRACT

The gut microbiota that lives in our large intestine has a close relationship with our health and well-being. Gut microbial composition depends on both environmental factors and factors from their hosts. This pilot study investigated dietary habits and fecal short-chain fatty acid (SCFA) concentration with substrates inulin and glucose in three ethnic groups (Caucasian, Chinese, Japanese) in Hawaii (n=9). Dietary intake was recorded for 3 to 4 days prior to fecal specimen collection. An in vitro fermentation was conducted to evaluate the energy production potential of the gut microbiota obtained from human fecal specimens. Caucasians had significantly higher food weight intake than the other two ethnic groups. With substrates added, Caucasians also produced a higher concentration of butyrate, propionate and total SCFA than Japanese subjects at time point 12 and 24 hr, which Japanese had significantly lower production ($P<0.05$). The SCFA concentrations produced by the individual subjects were widely variable over the 24-hour study period. Higher food weight, carbohydrate intake, and dietary fiber intake were associated with higher SCFA production in vitro. This study demonstrated that Caucasians had higher propionate, butyrate, and total SCFA production than Japanese subjects in response to added inulin. Further studies are required to determine if the effects of ethnicity and dietary pattern on bowel health are sustained in the long-term.

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CHAPTER 1

LITERATURE REVIEW

1. Introduction

The gut microbiota that lives in our large intestine has a close relationship with our health and well-being. Some evidence suggests that the gut microbiota plays a role in the development of obesity. There are growing concerns about obesity and its impact on human health. Obesity and overweight have been defined as abnormal or excessive fat accumulation that threatens health by World Health Organization (WHO 2013). Based on the WHO fact sheet, obesity has more than doubled worldwide since 1980. The estimated number of overweight adults was more than 1.4 billion, and at least 500 million were considered to be obese in 2008. Obesity is linked to higher mortality, worldwide. Overweight and obesity are major risk factors for a series of chronic diseases, including type II diabetes, cardiovascular diseases and certain cancers. At least 2.8 million adults die each year as a result of being overweight or obese (WHO 2012). Environment and lifestyle are two major contributors to the increase of obesity in recent decades (Bleich et al. 2008). Altered dietary intake not only affects the energy balance but also has a major impact on gut microbial composition, and the change of gut microbiota may promote obesity and increase the risk of developing metabolic diseases (Serino et al. 2012).

Bifidobacteria and lactobacilli are two members of gut microbiota with beneficial and health promoting properties (Suvarna & Boby, 2005). These bacteria

benefit hosts' health by producing short chain fatty acids (SCFA). Short chain fatty acids lower the gut pH and may inhibit gastrointestinal pathogens, (Gibson 1999) provide nutrients for the colonic epithelium, and may also contribute to prevent gastrointestinal diseases such as colon cancer, inflammatory bowel disease (IBD) and colitis (Cook & Sellin 1998). Recent studies found that the gut microbiota may affect to regulation of energy intake and influence the body weight. It has been hypothesized that long-term dietary changes can impact weight gain via changes in the gut microbiota. Gut microbial composition depends on both environmental factors and factors from their hosts. This review addresses the role played by the gastrointestinal microbiota in human health and disease, and the diversity of gut microbiota in different population groups.

2. Gut microbiota and its functions

The large intestine, also referred to as the colon, is the principal site of permanent microbial colonization in the human body. The colon contains a complex microbial ecosystem that contributes to many different metabolic functions (Macfarlane & Macfarlane 2012). It has been estimated that the gut microbiota in our bodies includes up to 200 common bacterial species and up to 1000 less common species. Recent studies suggest that the microbial functions include metabolism of xenobiotic compounds, amino acids, and carbohydrates (Greiner & Backhed 2011). The gut bacteria have a profound influence on their hosts' physiology and nutrition status (Qin et al. 2010). The gut microbes can contribute to energy harvest as an environmental

regulator of fat storage and adiposity, and changes of gut microbiota are associated with bowel diseases and obesity (Greiner & Backhed 2011). When the integrity of the bowel barrier is physically or functionally breached, gut bacteria can become pathogenic, resulting in infection and/or an inflammatory response. However, the constant interaction between the host and its microbial residents can bring important health benefits to the human host (Gourbeyre et al. 2011).

Probiotics have been defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001). In particular, species belonging to Bifidobacterium and Lactobacillus are the most widely used probiotic bacteria and are included in many functional foods and dietary supplements (Guarner et al. 2003). Many of these bacteria colonize the gut, and they can also alter the nutritional content of the foods in which they reside. For example, live bacteria (primarily Lactobacillus and some Bifidobacteria species) are used in yogurt to improve digestion of lactose and reduce symptoms of lactose intolerance in humans. This beneficial effect is due to the presence of microbial galactosidase (lactase) in the fermented milk product.

Prebiotics are one of the most common dietary methods to shape the species composition and metabolic activities of the gut microflora through diet. Prebiotics are defined as *non-digestible food ingredients (usually oligosaccharides, inulin) that are selectively utilized by one or more components of the beneficial gut microbiota and thus improves host health* (Gibson et al. 1995). Bacterial genera targeted for selective stimulation are the indigenous Bifidobacteria and Lactobacilli. Live bacteria are

essential for the effect of probiotics, since heated or pasteurized yoghurts could not prevent lactose malabsorption and symptoms of intolerance (Labayen et al. 2001). Prebiotics are a more convenient dietary intervention due to the diversity of food sources: functional foods such as beverages, granola bars, yogurt; supplements; natural sources. Both probiotics and prebiotics have been shown to prevent colon cancer in several animal studies (Burns & Rowland 2000). Evidence also suggests that probiotics and prebiotics may play a therapeutic role in the prevention of digestive diseases (Marteau 2001). But their role in reduction of risk of colon cancer in human subjects is not well established. Further studies are required to better establish their efficacy in human subjects.

The prevalence of obesity and related diseases such as type II diabetes, metabolic dysfunction and coronary heart disease (CHD) has vastly increased throughout the world. Recent studies have suggested that gut microbiota might be involved in the development of these disorders (Serino et al. 2012). Dietary composition and calorie intake appear to regulate intestinal microbial composition and function, and this will be described in next section. Studies have demonstrated that obesity and metabolic dysfunction may be associated with abnormal microbial changes, and microbiota may also play an important role in the treatment of these chronic diseases (Tilg et al. 2011).

3. Effects of diet on gut microbiota

Both animal and human studies have demonstrated that gut microbiota composition is altered in obese and diabetic populations (Murphy et al. 2012; Larsen et al. 2010). Macronutrient content of diet, as an environmental factor, not only contributes to body weight itself, but also influences the composition of gut microbiota. The gut microbiota is a dynamic microbiological system that can shift rapidly in response to altered diet. Previous studies have shown that a high-fat diet in mice shifts the gut microbiota and also causes damage to the gut mucosal barrier resulting in increased endotoxaemia and metabolic diseases (Cani et al. 2009). The plasma concentration of bacterial lipopolysaccharide (LPS), which is an inflammatory agent, increased during a fat-enriched diet, and resulted in metabolic endotoxemia (Cani et al. 2009). In their earlier published article, Cani et al. reported that although the reasons for the increase of LPS in plasma during high-fat feeding were still unknown, the LPS level was correlated with changes in intestinal microbiota. Furthermore, dietary fibers can reduce the impact of high-fat diets on the occurrence of metabolic diseases, and normalize plasma endotoxemia (Cani et al. 2007). These findings suggested that intestinal microbiota could be responsible for changes of metabolic endotoxemia and for the related diseases.

In their paper published in 2012, Serino et al. used a mouse model with the same genetic background to investigate whether the gut microbiota per se could influence different metabolic phenotypes, independent of changes in genetic background and diet

(Serino et al. 2012). The tested mice (n=100) were fed a high-fat carbohydrate-free diet (HFD) for three months and became either diabetic (HFD-D) or resisted diabetes (HFD-DR), at the same time, a comparison group of mice (n=10) was given the same diet as the test group, but had glucooligosaccharide added (HFD+GOS). They found that when these mice were switched to a high-fat carbohydrate-free diet from their regular diet (original diet not mentioned in the article), the gut microbiota composition was altered within 24 hours in all three groups (HFD-D, HFD-DR, HFD+GOS). Compared with diabetes resistant mice, the gut microbial profile in diabetic mice was associated with increased gut permeability. This was linked to increased endotoxaemia and increased adipose tissue inflammation. The results showed that the development of diabetes within a subgroup of mice was strongly associated with a change in the composition of the gut microbiota (Serino et al. 2012). To determine whether gut microbiota was the cause or consequence of the metabolic phenotypes, this study specifically modified it with dietary fibers (glucooligosaccharide). The results from the comparison group showed that the gut microbiota of mice fed dietary fiber was modified and prevented the occurrence of the diabetic phenotype. The authors concluded that the gut microbiota was altered by high-fat diet and showed a specific microbial signature of certain metabolic genotypes (Serino et al. 2012). Another study also used a mouse model to investigate the impact of gut microbiota on diet-induced obesity (Murphy et al. 2012). Two control groups were fed either a low-fat diet or a high-fat diet (diet-induced obesity), and the study group were fed a high-fat diet with vancomycin and bacteriocin-producing probiotic added. Vancomycin is an antibiotic

that selectively targets gram-positive bacteria. After twenty weeks, the diet-induced obese mice had a significantly higher proportion of Firmicutes and a lower proportion of Bacteroidetes compared with low-fat diet controls. For the vancomycin treated obesity group, the results showed significant reductions in the proportion of Firmicutes and Bacteroidetes and an increase in Proteobacteria. Those mice also gained less body weight and had lower fasting blood glucose and plasma tryglyceride levels compared with diet-induced obese controls, despite similar caloric intake (Murphy et al. 2012). These two studies both used mouse models to provide a confirmation for the role of gut microbiota in metabolic dysregulation and showed the association between gut microbiota composition and obesity. But these studies cannot be easily replicated in humans. In human subjects, the researchers cannot feed diets that be might harmful for the subjects' health. Although further research is still needed to investigate the effects of gut microbiota on the development of obesity and other metabolic disorders in human subjects, the animal studies demonstrate that dietary fiber can off-set other dietary influences on the gut microbiota.

In conclusion, the gut microbiota is a huge ecosystem living within our colonic lumen. Some of these bacteria are potentially harmful to the host, but the constant interaction between the host and its gut microbiota can bring important health benefits. It is now well established that diet, as an environmental factor, influences the composition of the gut microbiota, but little is known about the influence of the aging process on the status of gut microbiota. In the next section, previous studies that focused on metabolic characteristics of the gut microbiota from different age groups

will be reviewed.

4. Gut microbiota composition in different age groups

4.1 Infants

A recent study showed that the composition of gut microbiota among infants was related to the original pre-pregnancy weight and weight gain of their mothers during pregnancy (Collado et al. 2010). Higher body weights and BMIs ($\geq$ 25 kg/m^2) of mothers were related to higher concentrations of Bacteroides, Clostridium, and Staphylococcus and lower concentrations of the Bifidobacterium groups. However, mothers with normal weight and normal weight gain during pregnancy had babies with lower prevalences of *Akkermansia muciniphila*, *Clostridium difficile* and Staphylococcus groups (Collado et al. 2010). The gastrointestinal tract of newborn infants is colonized immediately after birth with environmental microorganisms, mainly from the mother. Biasucci et al. found that the mode of delivery strongly influences the composition of the intestinal microbiota within three days of life. Cesarean delivery babies were characterized by a substantial absence of Bifidobacteria species, with no or little influence of the type of feeding. On the other hand, vaginal delivered infants showed subject-specific microbial profiles, with some predominant groups such as *Bifidobacterium longum* and *Bifidobacterium catenulatum* (Biasucci et al. 2008).

Bifidobacteria have been identified as a typical beneficial component of the gut microbiota of healthy breast-fed infants. *Clostridia difficile* and *Staphylococcus aureus*

are two major gut bacteria that exist in allergic infants and in obese infants and adults (Collado et al. 2010). Akkermansia may cause mucus degradation that breaks the mucosal surface in the body of their hosts, resulting in inflammatory diseases and obesity (Collado et al. 2010). A recent study chose 44 Malawian 6-month-old infants and compared them with 31 children of the same age living in urban Finland to investigate the effect of food consumption among new born babies (Grzeskowiak et al. 2012). Both groups of infants were breast-fed and consumed age-appropriate diets based on the food supply related to the geographic region and culture. Bifidobacteria was the dominant microbial population in all of the infants, but the proportion in Malawian infants was greater than in Finnish infants (Grzeskowiak et al. 2012). *Bifidobacterium adolescentis*, *Clostridium perfringens*, and *Staphylococcus aureus* were absent in Malawian but detected in Finnish infants (Grzeskowiak et al. 2012). The possible reason that large amounts of Bifidobacterium, Bacteroides-Prevotella, and *Clostridium histolyticum* reside within the intestinal system of Malawian infants is the diet of the African children. Even though both Malawian and Finland infants were breast-fed, infants from Malawi were fed more plant polysaccharides, which are introduced together with breastfeeding along with seasonal varied foods (Grzeskowiak et al. 2012). Maintenance of a healthy gut microbiota composition may be necessary for newborn babies all over the world. Beneficial bacteria were altered by the environmental exposures, including diet, delivery methods and health status of mothers. Further research is needed to investigate the interrelations between diet and the composition of gut microbiota as well as the role of the microbiota in preventing

diseases on newborn infants.

4.2 Children and Adolescents

As children grow up, dietary habits and environmental exposure are the main factors contributing to the diversity of human gut microbiota (Backhed F et al. 2005). The prevalence of allergy, autoimmune disorders, and inflammatory bowel disease (IBD) both in adults and in children has increased in recent decades (Okada et al. 2010). It is hypothesized that improvements in hygiene, together with decreased environmental microbial exposure in childhood, are responsible for this increase (Braun-Fahrlander 2003). The gut microbiota plays a crucial role in the pathogenesis of IBD, and the gut microbial status and bacterial types are different in children with and without a predisposition to allergy (Braun-Fahrlander 2003).

Recent studies demonstrate that obesity is associated with imbalance in the normal gut microbiota (Turnbaugh et al. 2009). Childhood obesity is associated with increased incidence of obesity and/or type II diabetes in adulthood (Karlsson et al. 2012). A case-control study in south Sweden with children (4-5 years old) concluded that compared to the control group, the obese group was found to have significantly more Enterobacteriaceae and less Desulfovibrio and *A. muciniphila*-like bacteria. The diversity of the intestinal microbiota was lower for the obese/overweight group (Karlsson et al. 2012). This study also found Enterobacteriaceae was at significantly higher concentrations in obese/overweight children compared to individuals with body weight within the normal range (Karlsson et al. 2012). As one of the gram-negatives, Enterobacteriaceae are potent stimulators of inflammation because of the

lipopolysaccharides (LPS) in their outer membrane, and the *A. muciniphila* can act on the degradation of mucin and is commonly found in the human gastrointestinal tract (van Passel et al. 2011). Childhood overweight and obesity are highly prevalent in the United States, affecting one-third of children and adolescents. Compared with normal-weight children, overweight children and adolescents are at greater risk for health problems and are more likely to become obese adults (Wang et al. 2013). Based on the association of gut microbiota and childhood overweight/obesity, future research should focus on the microbial ecosystem and its impact on health during childhood and later in life.

4.3 Women

As stated previously, the composition of gut microbiota on newborn babies was associated with the health status of their mothers. Villamor et al. concluded that overweight pregnant women have a higher chance to deliver higher birth weight babies (2006). They also found that a gain of 3 or more BMI units per se was significantly associated with the risk of stillbirth (Villamor et al. 2006). Furthermore, excessive weight gain during pregnancy is associated with deteriorated glucose tolerance and increased risk of gestational diabetes (Chu et al. 2007). Previous studies linked gut microbiota composition to body weight, and it also may affect the mother's weight gain during pregnancy (Collado et al. 2008). The *Bacteroides* group might play an important role in energy storage and weight gain because of its high presence in samples from obese women and also a high presence in women showing excessive weight gain during

pregnancy (Collado et al. 2008). In addition, Collado et al. suggested that a high concentration of *S. aureus* may be associated with inflammatory processes and also with fat storage in overweight mothers (Collado et al. 2008). Women with excessive weight gain also have significantly higher amounts of *Escherichia coli* compared with women with normal weight gain over pregnancy. Numbers of *A. muciniphila* and Bifidobacterium, which are beneficial gut bacteria, were higher in women with normal weight gain than in those with excessive weight gain (Santacruz et al. 2010).

Most of the human studies that have been reviewed focused on the differences in gut microbiota between obese and lean subjects, but it's also important to investigate whether there are differences in the gut microbiota composition in overweight/obese subjects with and without metabolic disorders. Although men and women had a similar prevalence of metabolic syndrome among whites and other ethnic group, women in some specific ethnic groups had a significantly higher prevalence of metabolic disorder (Ford et al. 2002). In the United States, African American women had about a 57% higher prevalence of metabolic disorder than men did; Mexican American women had about a 26% higher prevalence than men did (Ford et al. 2002). A study on overweight/obese women with or without metabolic disorder, and normal weight women was conducted to investigate the differences of their gut microbiota status. The authors found that certain members of *Eubacterium rectale-Clostridium coccoides* group were associated with obesity-related metabolic disorders, but not obesity per se. (Munukka et al. 2012).

4.4 Seniors

The composition of the human intestinal microbiota is individual-specific and stable over time in healthy adults (Claesson et al. 2012). But the composition of the intestinal microbiota in older people displays more inter-individual variation than that of younger adults (Claesson et al. 2011). Recent studies suggested that elders have a decrease in anaerobes and Bifidobacteria and an increase in Enterobacteria (Hebuterne 2003). Andrieux et al. demonstrated that elderly humans had higher concentrations of metabolites from protein fermentation (ammonia, valerate, isobutyrate, and isovalerate) compared with younger adults and children (Andrieux et al. 2002). Higher end-products of protein fermentation in the human colon may cause uncomfortable bowel movements, and they are also cytotoxic at high concentrations (Ramakrishna et al. 1991). Possible factors that affect the intestinal microbiota upon aging include deterioration in dentition, salivary function, digestion and intestinal transit time (Claesson et al. 2011). Diet as one of the environmental factors has been shown to influence gut bacteria composition in animal models and small human studies (previous references). The effects of age and diet on intestinal morphology and colon health are of importance. Especially for elders, differences in gut microbiota composition are shaped by diet which then affect the health of the hosts (Claesson et al. 2011).

5. Short-Chain Fatty Acids (SCFA)

Short-chain fatty acids are a sub-group of fatty acids with aliphatic tails of two to six carbons (Brody 1999). The short-chain fatty acids include primarily acetic, propionic and butyric acids. Short-chain fatty acids are one of the principal metabolites of fermentable fibers, and different fibers are fermented to different short-chain fatty acids in different amounts by different gut bacteria (Gropper et al. 2009). For example, pectin resulted in higher concentrations of propionate in proximal and distal colon, whereas wheat bran resulted in a higher concentration of butyrate (Lupton & Kurtz 1993). Bifidobacteria can produce acetate and lactate from pectin fermentation (Gropper et al. 2009). Meanwhile, butyrate and propionate can also converted to SCFA acetate or lactate through cross-feeding by other bacteria (Belenguer et al. 2006). Functions of SCFAs include lowering gut pH, inhibition of gastrointestinal pathogens (Gibson 1999), feeding colonic epithelium as nutrients after absorption by the colonocyte (Cook & Sellin 1998), and may also contribute to the prevention of gastrointestinal diseases such as colon cancer, inflammatory bowel disease and colitis (Galvez et al. 2005).

Short-chain fatty acids may also affect body mass by influencing the secretion of gut hormones involved in food intake regulation. Glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and oxyntomodulin have been proposed as important modulators of appetite (Stanley et al. 2004). Among those peptides, GLP-1 is also involved in the regulation of pancreatic secretion of insulin and in the differentiation and maturation of pancreatic beta cells (Delzenne et al. 2005). Changes in gut hormones have been

correlated with increased SCFA concentrations. However, the increased dietary fiber in the test diets correlated with a decrease in energy intake, which is a confounding factor. Delzenne et al. demonstrated that gut hormones can be regulated by high-fiber diet and showed reduced weight or improved glucose homeostasis by using rat model (Delzenne et al. 2005). Rats received either a standard diet or the same diet added with oligofructose for 3 weeks. The addition of oligofructose to the diet increased the GLP-1 secretion. The mechanism may be due to increased production of the precursor proglucan mRNA (Delzenne et al. 2005). However, these rats also consumed a lower energy diet when oligofructose was added, so it's unclear whether these results were truly from dietary fiber fermentation.

Previous animal studies suggested that specific SCFA played an important role in the regulation of the expression of the intestinal proglucagon gene (Drozdowski et al. 2002). A study on healthy human volunteers was conducted to investigate whether SCFAs affect proximal gut motility and gastric tone (Ropert et al. 1996). Six healthy volunteers were studied after oral administration of 20 g lactulose and intracolonic infusions of 20 g lactose and SCFAs. Gastric tone and peripheral intestinal peptide levels were measured. Short-chain fatty acid exposure, both from lactulose fermentation and intracolonic infusion, decreased gastric tone, but GLP-1 and PYY concentrations did not change significantly compared to saline control (Ropert et al. 1996). The influence of SCFA exposure on gut peptides needs further research.

6. Branched-Chain Fatty Acids

Human colonic bacteria are capable of protein breakdown and amino acid fermentation. Branched chain fatty acids (BCFA) are formed solely from proteins and amino acids. Putrefactive compounds resulting from microbial fermentation of undigested amino acids include ammonia, phenols, indoles, and branched-chain fatty acids (Montagne et al. 2003). These end-products of amino acid fermentation result in uncomfortable bowel movements and are also cytotoxic at high concentrations (Ramakrishna et al. 1991). The presence of branched chain fatty acids confirms the occurrence of proteolysis in human colon and has been linked to disease states in humans (Nyangale et al. 2012). The BCFA concentration may be critically important in determining large bowel function particularly in those people living on diets low in fermentable carbohydrate.

7. Gut microbiota composition vs. obesity: genetics or environment?

Excessive energy intake and low energy expenditure are the primary causes of obesity, but it is well established that certain individuals have a genetic predisposition for excess body fat. The gut microbiota may be a contributor to this genetic predisposition. The presence of a gut microbiota is linked with increased body fat, as demonstrated in mice (Backhed, 2004). Mice were raised in a germ-free (GF) environment and lacked a gut microbiota. One group of GF was “conventionalized” by exposure to fecal matter from conventional mice, thus developing a gut-microbiota. The conventionalized GF mice experienced a 60% increase in body fat within 10 to 14

days, despite an associated decrease in food consumption (Backhed et al. 2004).

To better understand this phenomenon, adult GF mice were colonized with a microbiota harvested from the obese or lean mice (Turnbaugh et al. 2006). The microbiota of obese donor had a greater amount of Firmicutes compared with the lean donor, and mice colonized with an obese microbiota gained a significantly greater percentage of body fat over two weeks than mice colonized with microbiota from lean donors (Turnbaugh et al. 2006). Additionally, cecal SCFA content was greater in obese mice compared to lean mice, which suggests increased energy production by the gut microbiota (Turnbaugh et al. 2006). Gut microbiota affects energy harvest and energy storage in the host as an important environmental factor. Even though the alteration in efficiency of energy harvest from gut microbiota was not a great contribution towards total energy intake, over time, it could result in significant changes in body weight (Flegal et al. 2000).

However, not all studies have demonstrated that the gut microbiota affect weight gain. Fleissner et al. conducted an experiment using GF mice to define the exact role of the intestinal microbiota in diet-induced obesity. Germ-free mice and conventional mice (CV) were fed either a semi-synthetic low-fat diet (LFD) or a semi-synthetic high-fat diet (HFD) for 4 weeks. Germ-free mice fed the HFD gained significantly more body fat and body weight than CV mice fed the same food. The mean body weight of the GF mice was significantly higher than that of CV mice prior to the start of the test diets, which confounds these findings. The changes in weight gain contradicts the findings of Backhed et al (2004), who demonstrated that the CV mice gained

significantly more body weight and body fat than the GF mice. Diet significantly affected the proportions of bacteria groups present, with HFD decreasing total bacteria and bacterial diversity more than LFD.

In their second experiment, Fleissner et al. (2010) compared the effects of HFD and a Western-style, high-fat, sugar-rich diet (WD) on weight gain and body fat of GF and CV mice. Interestingly, GF mice fed the WD had 41% less body fat than CV mice fed the same diet, but GF mice in the HFD group had similar body fat content to that of CV mice also fed HFD. This suggests that diet may play a more pivotal role than the composition of the gut microbiota. The fecal microbiota analysis showed that Bacteroides and Firmcutes were higher at baseline than during the intervention with HFD or WD in the CV mice. Although the macronutrient compositions were similar among HFD and WD, but they differ in the type of carbohydrates and lipids, which may explain the differences in fat gain between the two treatments. Another potential barrier in experiment 2 was at this time, CV mice had significantly higher body weight at the start of intervention than GF mice.

The third experiment was conducted to investigate the effects of diet on the gut microbiota. The comparison was among three groups of CV mice fed either one of the three diets: Standard chow diet (SD), HFD, or WD for 4 weeks. After 4 weeks, the mice fed HFD and WD gained significantly higher body weight and body fat than those of the mice fed the SD. The majority gut microbiota in all three groups was Bacteroidetes and Firmcutes, but mice on HFD and WD harbored additional Proteobacteria and Deferribacteriaceae. The percentage of Bacteroidetes on the HFD and WD was

significantly lower than on the SD. In conclusion, the findings of Fleissner et al. (2010) conflicted with the findings of Backhed et al. (2004). Fleissner et al. (2010) reported that germ-free mice were not generally protected against diet-induced obesity and although HFD led to a dramatic reduction in microbial diversity. The effect of the HFD cannot be attributed to the crude fat content alone. The gut microbiota is a sensitive ecosystem that has a symbiotic relationship with its host. Different study materials, identification methods, and the body status of study subjects all be able to affect the results. The exact role of gut microbiota in host energy metabolism and obesity is still obscure, and deserves further research.

Observational and intervention studies in humans have demonstrated an association between gut microbiota and body fat. Obese humans have a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria compared with lean individuals (Turnbaugh et al. 2009), and the Bacteroidetes progressively increased during weight loss (Ley et al. 2006). Monozygotic and dizygotic twins provided a model for assessing the impact of genotype and shared early environmental exposures on the gut microbiome. Turnbaugh et al. (2009) conducted a study on adult female monozygotic and dizygotic twin pairs (concordant for leanness or obesity), and their mothers, to address how host genotype, environmental exposure and host adiposity influence the gut microbiota communities (Turnbaugh et al. 2009). The general characteristics of the human gut microbiome were shared among family members, but each person had their own gut unique microbial community. The most important finding was that obesity was associated with significant decrease in bacterial diversity.

The decreased diversity altered the representation of bacterial genes and the metabolic pathways that may contribute to the obese state (Turnbaugh et al. 2009).

As stated previously, the composition of the human gut microbiota undergoes dramatic changes during postnatal development. The adult gut microbiota community is influenced by environmental factors as well as by host genotype. The composition of the gut microbiota remains relatively stable over time when a steady state is reached, reflecting that no major changes in lifestyle or environment occur (Round et al 2009). There were few reports based on adequate subject numbers to investigate the intra-subject variation thus to address the effect of geographic origin on the human gut microbiota. Although there were some reports comparing gut microbiota composition among European countries (previous reference), between rural African and European children (previous reference), there are no data available on the differences between the gut microbiota from different ethnicities in a similar geographic area.

The gut microbiota influences human health, and we know that each individual harbors a distinct community of bacteria. One mechanism by which the gut bacteria influence host health and energy uptake is through fermentation of dietary fiber. However, individual variability in dietary fiber fermentation is poorly understood. This is an area that needs further research so we can better understand the role of gut bacteria in human health.

CHAPTER 2

METABOLIC POTENTIAL OF GUT MICROBIOTA IN HUMAN SUBJECTS FROM THREE ETHNIC GROUPS IN HAWAI'I

1. Introduction

Human health is influenced by gut microbiota through its effect on the gut defense barrier, immune development, and nutrient utilization (Hooper & Gordon 2001). Many species of bacteria have evolved and adapted to live and grow in the human colon, and the constant interaction between those bacteria and their host can infer important health benefits (Salminen et al. 1998). The main functions of gut microbiota include metabolism of residual dietary components, trophic functions, and protective functions (Roberfroid et al. 1995). Lactobacilli and Bifidobacteria are two of the best studied beneficial bacteria because of their saccharolytic fermentation (Salminen 2001). These bacteria are also the most widely used in many functional foods and dietary supplements as probiotics (Guarner et al. 2003). Bifidobacteria and Lactobacilli produce acetate and lactate which can be converted to butyrate and propionate through cross-feeding by other bacteria (Beards et al. 2010). Short-chain fatty acids (SCFA) include primarily acetic, propionic, and butyric acids. They are the principal resulting metabolites of fermentable fibers, and different fibers are fermented to different short-chain fatty acids in different amounts by different gut bacteria (Gropper et al. 2009). Short-chain fatty acids are important for colonic function and mucosal health. Short-chain fatty acids lower luminal pH, and thus may inhibit gastrointestinal pathogens (Gibson 1999). They also act as preferred sources of energy

for the colonic epithelium and may be utilized directly by colonic epithelial cells (McOrist et al. 2008).

Gut microbiota is a large ecosystem living within our colonic lumen. It is now well established that diet, as an environmental factor, influences the composition of the gut microbiota (Cani et al. 2007; Serino et al. 2012). However, ethnicity which includes genetic aspects as well as dietary aspects may also impact the composition of the gut microbiota. Functionally, the presence of different bacteria groups changes the rate and amount of SCFA production when dietary fibers reach the colon. These differences in SCFA production, along with differences in other bacterial influences (LPS uptake, immune balance) may influence the predisposition for chronic disease and weight gain (Mueller et al. 2006). This pilot study explores daily dietary habits and SCFA concentrations after *in* vitro fermentation of fecal matter from in nine healthy human volunteers from three different ethnic groups (Caucasian, Chinese and Japanese) in Hawai'i. The aim of this study was to establish associations among gut microbiota composition, diet, and ethnicity, as a mechanism for ethnicity-based health concerns.

2. Materials and Methods

2.1. Subject recruitment

This study was approved by the University of Hawai'i at Manoa IRB Human Subjects Committee. Female subjects aged 40-55 were recruited via advertisements posted in the Honolulu area. We chose women in this age group since gut SCFA production in the female population has rarely been studied and as adults, glycolytic

activities appeared less variable with more stable gut microbiota composition than people in a younger or older age (Andrieux et al. 2002). Subjects were screened via telephone or email. Eligible subjects provided informed consent and attended a study visit to receive study supplies and instructions for completing the diet record and collecting the fecal specimen. Subjects completed a diet record for 3-4 days prior to fecal collection. On the third or fourth day, the subjects collected a fecal specimen using the Commode Specimen Collection device. Subjects sealed the fecal specimen in a plastic anaerobic bag containing an AnaeroPouch ® (Mistubishi Gas Company, Tokyo, Japan) to generate anaerobic conditions. The subjects placed the specimen on ice and delivered the specimen to the University of Hawai'i at Manoa immediately. Upon delivery, subjects received compensation for participating in the study.

2.2. Substrates and reagents

The substrates used in this study were all ingredients used widely in nutrition science research. The substrates were inulin (Sweet Fiber® Purpose Foods, Pacific Palisades, CA< USA) which is a known highly fermented dietary fiber, glucose ($C_6H_{12}O_6$, 99% purity, Fisher Scientific, Pittsburgh, PA, USA) was used as a positive control. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3 In vitro fermentation

The *in vitro* fermentation method was previously described by McBurney and Thompson was used (1989). Inulin and glucose (0.5 g for each substrate) were weighed into autoclaved 50-ml serum bottles and 40 ml autoclaved sterile trypticase peptone media was added to each bottle. Both inulin and glucose were fully dissolved. One liter

of nutritive media contained the following reagents:

2.49 g trypticase peptone

1.0 g ammonium bicarbonate

8.75 g sodium bicarbonate

1.43 g anhydrous sodium phosphate

1.55 g anhydrous potassium phosphate monobasic

0.6 g magnesium sulfate

0.12 mg reaszurin

1.12 mmol calcium chloride

0.63 mmol manganous chloride

0.15 mmol cobalt chloride

0.04 mmol ferric chloride

Reducing solution was prepared with the following reagents:

950 ml distilled water

6.25 g cysteine hydrochloride

40 ml 1N NaOH

6.25 g sodium sulfide nonahydrate

Fecal slurry was prepared using fresh feces from healthy donors (who had not taken antibiotics for 6 months beforehand). Fecal samples were diluted with PBS at a ratio of 1:6. Reducing solution was added to the fecal inoculum to obtain a ratio of 2:15. The serum bottles were inoculated with 10 ml of the slurry along with 1.0 ml Oxyrase ®

oxygen reducing enzyme (Oxyrase Inc., Mansfied, OH). The bottles were flushed with CO_2 gas (carbon dioxide) immediately to eliminate oxygen. The bottles were mixed and sealed, and were gently shaken in a 37°C water bath. Samples were removed from the serum bottles at 0, 4, 8, 12 and 24 h fermentation for analysis. Copper sulfate (1 ml, 200 g/L) was added to each bottle to cease fermentation, and 2 ml samples were taken for SCFA analysis.

2.4 Short-chain fatty acid analysis

The method was carried out as described by Zhao et al. with modifications (Zhao et al 2006). Samples were centrifuged (3000x g for 10 min) at room temperature to remove particulate material. Supernatant (0.75 ml) was transferred into a micro-centrifuge tube and vortexed with 0.3 ml 25% meta-phosphoric acid to precipitate protein, and then the sample was incubated at 4°C for 30 minutes and centrifuged for 15 minutes at 5000x g at 4°C. The supernatant was transferred to another micro-centrifuge tube and frozen at -80°C over night.

Before transferring to the GC vial, the samples were briefly vortexed, and filtered through a 0.45 µm nylon filter. Total SCFA, acetate, propionate, and butyrate were quantified via gas chromatography (Hewlett-Packard 5890 gas chromatograph, Restek 30m Stabilwax-DA column, 530µm ID, injector temperature 200°C, detector temperature 250°C, initial oven temperature 80°C, increased by 10°C /min to 145°C, then 70°C /min to 200°C). The carrier gas was He at a column flow rate of 12 ml/min with a split ratio of 1:12. Ethyl butyrate served as the internal standard. Concentration of SCFA was expresses as µmol/g feces.

2.5 Diet analysis

Nutrition Database Software for Research (NDS-R, Nutrition Consulting Center, Minneapolis, MN) was used to analyze the 4-day diet records. Total energy intake, total carbohydrate, total fat, total protein, pectin, alcohol, water, and dietary fiber (soluble and insoluble) were calculated for each day of intake.

2.6 Statistical analysis

Differences among SCFA concentrations at 0, 4, 8, 12 and 24h fermentation for each substrate were tested for significance using ANOVA with Student-Newman-Keuls pair wise comparison. Differences were considered significant if $P<0.05$.

3. Results

3.1 Study subjects

As shown in Table 1, all the subjects (n=9) were healthy females; the age range was from 40 to 51 years, and the average age was 45 years old. BMI ranged from 19 to 24.2 with average BMI 23.1; none of the subjects were obese. All subjects consumed their regular diets and completed a 4-day diet record for the duration of the study and provided fresh fecal specimens on days 3 or 4 for analysis. None of the subjects were smokers and did not use antibiotics within 6 months. Subject 19, 77, 87 and 64 were Caucasian; 70, 74, 23 were Chinese; 76 and 52 were Japanese.

3.2 Dietary analysis

The mean daily intakes of total energy, total carbohydrates, protein, fat, dietary fiber, soluble fiber, insoluble fiber, pectin and alcohol were similar among the three

ethnicity groups (Table 2). The Caucasian group had a significantly higher amount of food weight and water consumption than other two groups ($P<0.05$). All the measured outcomes were significantly different among subjects (Table 3). Subject 74 and 76 both had a significantly lower food weight account than others, and 74 also had lowest daily calorie consumption as well as subject 64. In contrast, subject 87 had the highest food weight, energy, carbohydrate, and total dietary fiber intake. Subject 19 had the highest intake of soluble fiber; subject 64, 70, and 74 had lower intake than others. As stated previously, diet as an environmental factor, not only contributes to body weight itself, but also influences the composition of gut microbiota. The gut microbiota is a dynamic microbiological system that shifts rapidly in response to altered diet. The SCFA are produced solely via bacterial anaerobic fermentation in the colon. Dietary intake can also influence SCFA production depending on gut microbiota composition.

3.3 SCFA concentration analysis by subject

The SCFA concentrations during *in vitro* fermentation measured by GC are shown in Tables 4-6. In the tested samples with no fiber added (Table 4), subject 77 had significantly higher concentration of acetate, butyrate and total SCFA at time point 8 hours ($P<0.05$). With glucose added (Table 5), subject 70 and 77 both had higher concentrations of acetate, propionate, butyrate and total SCFA at time point 4 hours ($P<0.05$). And in the inulin group (Table 6), subject 87 had higher acetate, propionate, butyrate and total SCFA level than others at time point 0 hour($P<0.05$). But by the end of the fermentation (24 hour), subject 19 had the highest concentration of all three SCFAs and also higher total SCFA level than others with inulin added ($P<0.05$).

For acetate production, by the end of the fermentation, subject 52 became the highest producer with glucose added, and subject 23 and 76 were the lowest. With inulin added, subject 19 and 70 produced more acetate at time point 24 hour. There wereno significant differences between subjects for propionate concentration in the blank group. But with glucose added, subjects 19 and 52 had a significantly greater level of propionate concentration by the end of the fermentation. With inulin added, subject 19 was the biggest propionate producer at 24 hours, and subjects 23, 52 and 76 were significantly lower than others. Subjects 19 and 52 had higher concentrations of total SCFA than others with glucose added at time point 24 hour, whereas subject 23 and 76 were had lower concentrations. With inulin added, subject 19 had the highest concentration of total SCFA through the end of the fermentation, subject 70 also had a higher total SCFA concentration, but subject 23, 52 and 76 had significantly lower production than others.

3.4 SCFA concentration analysis by ethnicity

No significant difference was found between the groups with no fiber added (Table 7). With glucose added, the Caucasian group had a significantly higher butyrate production than Chinese and Japanese at time point 12 hr ($P<0.05$) (Table 8). With inulin added, Caucasians produced a higher concentration of butyrate at time point 24 hr than Japanese subjects ($P<0.05$) (Table 9). For propionate and total SCFA production, both Caucasians and Chinese had significantly higher concentrations than Japanese at time point 24hr ($P<0.05$) (Table 9).

4. Discussion

Gut SCFA production in the female population has rarely been studied. As adults, glycolytic activities appeared less variable with stable gut microbiota composition (Andrieux et al. 2002). In our study, three ethnic groups showed a similar pattern of dietary intake, except Caucasians had significantly higher food weight and water intake than the other two groups, but with no difference among daily calories, total carbohydrates, proteins etc. A plausible explanation for this could be a greater amount of liquid intake such as coffee and/or water. The Caucasian group also had significantly greater butyrate production with glucose and inulin added. Diet did not show a strong effect on gut microbiota composition which has been reflected by the SCFA concentration level based on the ethnic group data, but possible mechanism could be ethnic based gut microbiota difference. SCFA formation indicated that the substrates added to the fermenters were being utilized by the gut microbiota. Butyrate acts as an important energy source for colonocytes and may prevent colon cancer (Cook & Sellin 1998; Galvez et al. 2005). A recent study reported that representative butyrate-producing anaerobes belonging to the Gram-positive Firmicutes families Lachnospiraceae and Ruminococcaceae (Scott et al. 2013). The result of butyrate production from the fermentation of glucose and inulin may reflect a higher amount of this specific microbiota in the Caucasian group. Further definition test such as RT-PCR of gut microbiota is required to determine the proportion of the microbial composition. When we looked at individual data, the fecal SCFA concentrations were widely variable over the 24-hour study period. One of the Caucasian participants, Subject 87

not only had the highest total carbohydrates and total fiber intake, but also had higher acetate, propionate, butyrate and total SCFA production with inulin added to the fermentation at time point 0. High food weight and carbohydrate intake presumably results in a larger amount of food residue reaches the colon from the small intestine. The amount of the food residue, especially undigested carbohydrate (dietary fiber), has a major effect on colonic function primarily through its interaction with gut microbiota. Although this subject also had the highest fat intake, but higher amount of dietary fibers can reduce the impact of high-fat diet on the occurrence of the metabolic disease, based on the research by Cani et al. (Cani et al. 2007). Dietary fiber is the most important dietary substrate for the colonic microflora and in accord with the observed correlation of fiber intake, fermentation increased on the higher fiber intake as reflected by the higher fecal concentrations of total and the major individual SCFA.

Duncan et al. showed a significant reduction in fecal SCFA concentration in response to a low-carbohydrate compared with a high-carbohydrate diet in obese subjects that correlated with the level of carbohydrate intake (Duncan et al. 2007). This finding also reflected in our study. Subject 76 had a lower total fiber, soluble fiber and insoluble fiber intake, also had a lower SCFA concentration in all three tested SCFAs and total SCFA product. As stated before, SCFA is important for maintaining normal bowel function and prevent colonic diseases (Gibson 1999). Fecal SCFA concentration and excretion lower on these subjects may suggest that their dietary behavior may have adverse consequences for gut health in the future. Higher food residue also associated with increased stool bulk which is associated with intestinal motility and causing more

frequent bowel movements and shorter colonic transit time (Brinkworth et al. 2009). It benefits to prevent colonic cancer by reduce exposure of the colonic epithelium to potentially harmful agents in the human stool such as mutagens, carcinogens and procarcinogens (Topping & Clifton 2001).

A recent study found that the gut microbiota of infants in Southeastern Africa differs significantly from that in a Northern European country (Grzeskowiak et al. 2012). The author found out that the Bifidobacteria which is major acetate and lactate producer was dominant microbial population in all of the infants, but the proportion in Malawian infants was greater than in Finnish infants. They concluded that the possible is the diet of the African children. Even though both Malawian and Finland infants were breast-fed, infants from Malawia were fed more plant polysaccharides, which are introduced together with breastfeeding in addition with seasonal varied foods (Grzeskowiak et al. 2012). The composition of the human gut microbiota undergoes dramatic changes during postnatal development. The adult gut microbiota community is influenced by environmental factors as well as by host genotype. Subject 19, 77 and 87 were from Caucasian group in our study, but they all reacted well with added ingredients and had significantly higher SCFA concentration than others through the study (Figure 1-4). Beneficial bacteria were altered by the environmental factors, diet is the most important one for adults. Hawai'i is one of the most racially diverse states in the United States. In the 2010 Census Interactive Population Search, resident of Hawai'i includes 38.6% Asian, 24.7% White. Japanese and Chinese are two major groups for Asian population in Hawai'i. Ethnic groups in the United States have

different dietary cultures and rates of chronic disease (National survey 2002). Within the unique cultural environment of Hawai'i, in addition to a long Asian immigration history, the eating habit for Caucasians that lives in Hawai'i may shift to an Asian lifestyle including increased intakes of total carbohydrate and dietary fiber. To study this, a further cohort study is needed.

The protein Recommended Dietary Allowances (RDA) is 0.8 g per kg of body weight. The daily protein intakes from three ethnic groups were all higher than the RDA recommendation. The influence of high intake of protein should also be considered. Branched chain fatty acids (BCFA) are formed solely from proteins and amino acids. Putrefactive compounds of microbial fermentation of undigested amino acids include ammonia, phenols, indoles, and branched-chain fatty acids (Montagne et al. 2003). Those end-products of protein fermentation in human colon not only result in uncomfortable bowel movement, but are also cytotoxic at high concentrations (Ramakrishna et al. 1991). The presence of branched chain fatty acids confirms the occurrence of proteolysis in human colon and has been linked to disease states in humans (Nyangale et al. 2012). The BCFA concentration may be critically important in determining large bowel function particularly in those people living on diets low in fermentable carbohydrate.

Compared to the same ethnic group living in the country of origin, Japanese, Chinese, Koreans and Filipino that live in Hawai'i have much higher prevalence of diabetes the prevalence of diabetes (Abate & Chandalia 2003). Within Hawai'i residents, Japanese and Native Hawai'ian have a higher risk of developing type 2

diabetes during weight gain than Caucasians (Morimoto et al. 2011). Japanese also showed a higher risk both on colorectal cancer and rectal cancer than Caucasian in Hawai'i (Ollberding et al. 2010). While these health status concerns have been well documented, mechanisms behind the increased prevalence of chronic disease in some specific ethnic groups remain unclear. So far only few studies have investigated the metabolic potential of gut microbiota in different ethnic groups. This study examined the dietary habit and fecal SCFA concentration in three major ethnic groups live in Hawai'i. In order to achieve a realistic view of their normal life, we did not apply a controlled diet but recorded dietary intake and composition as a study variable.

As a pilot study, some limitations were also obvious. Small sample size may have profound effects on the outcome and worth of the study. Another weakness is that we did not get chance to investigate the local population such as Local Hawai'ian and Pacific Islanders because of limited access and no-responding bias. Self-estimate bias may be another factor that influenced the result. When looked back to their dietary record, we discovered that subject 70 had some incorrect measurement about her daily intake either on food description or weight measurement. Subject 77 is a consumed a large amount of coffee, which may also influence her gut microbiota composition. This may explained why these two subjects had such lower consumption of fiber intake, but still in a higher end of SCFA concentration. For future studies, we would like to combine the bowel habit data and run the bacteria identification process to get a more completed view of metabolic potential of gut microbiota in different ethnic groups in Hawai'i. Further studies are required to determine if the effects of dietary pattern on

bowel health are sustained in the long term and how to promote healthy lifestyle thus improve colonic health in high-risk populations.

6. Data Tables

Table 1. Characteristics of the female subjects in three ethnic groups

Subject	**19**	**64**	**77**	**87**	**23**	**70**	**74**	**52**	**76**
Age (years)	43	41	51	45	43	51	41	40	50
Weight (kg)	69	66	66	74	64	54	48	59	66
Height (cm)	174	174	171	180	165	152	162	162	165
BMI (kg/m^2)	23.8	22.9	23.4	24.2	24	23.4	19	23	24
Ethnicity	Caucasian1	Caucasian2	Caucasian3	Caucasian4	Chinese1	Chinese2	Chinese3	Japanese1	Japanese2

Table 2. Daily dietary intake by ethnicity

	Caucasian	Chinese	Japanese	P-value
Weight (g)	4156±352^{A}	2422±336^{B}	2315±302^{B}	0.0006
Energy (Kcal)	1686±211	1761±200	2074±213	0.4682
Total Carb (g)	190±30	208±19	199±31	0.8864
Total Protein (g)	66±9	84±12	101±14	0.1232
Protein (g/kg body weight)	1.0	1.5	1.6	
Total Fat (g)	70±9	61±10	81±9	0.4433
Total Dietary Fiber (g)	24±3	17±2	21±4	0.1200
Soluble Fiber (g)	9±1	6±1	6±2	0.0836
Insoluble Fiber (g)	15±2	11±1	14±2	0.2535
Pectin (g)	3.1±0.6	3.4±0.5	2.6±0.7	0.7008
Alcohol (g)	12±5	9±5	25±4	0.1017
Water (g)	3824.0±344.5^{A}	2066.4±304.6^{B}	1912.0±250.5^{B}	0.0002

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

Table 3. Daily dietary intake by subject

	CA 1	CA 2	CA 3	CA 4	CH 1	CH 2	CH 3	JP 1	JP 2	P-value
Weight (g)	3494±332^{ABC}	4389±234^{AB}	3869±178^{ABC}	4890±1700^{A}	3693±293^{ABC}	2358±318^{BCD}	1215±164^{D}	2804±455^{ABCD}	1827±245^{DC}	0.0005
Energy (Kcal)	1862±192^{A}	842±95^{C}	1509±53^{AC}	2874±278^{B}	2235±118^{AB}	2071±304^{AB}	977±146^{C}	2384±312^{AB}	1765±223^{A}	<0.0001
Total Carb (g)	211±26^{ACD}	111±8^{D}	115±11^{D}	376±32^{B}	276±6^{A}	179±22^{CD}	170±32^{CD}	261±37^{AC}	137±25^{D}	<0.0001
Total Protein (g)	101±20^{A}	30±4^{B}	66±8^{AB}	78±17^{AB}	103±12^{A}	117±9^{A}	33±2^{B}	119±22^{A}	83±16^{AB}	0.0002
Protein (g/kg bodyweight)	1.5	0.5	1.0	1.1	1.6	2.2	0.7	2.0	1.3	
Total Fat (g)	73±7^{AB}	37±9^{BC}	64±6^{B}	121±18^{A}	86±13^{AB}	77±14^{AB}	22±2^{C}	86±13^{AB}	77±15^{AB}	0.0003
Total Dietary Fiber (g)	27±3^{AB}	25±5^{ABC}	15±2^{BCD}	33±7^{A}	25±2^{ABC}	11±2^{D}	14±2^{CD}	30±3^{A}	12±3^{CD}	<0.0001
Soluble Fiber (g)	12±1^{A}	8±2^{AB}	8±1^{A}	9±1^{A}	10±1^{A}	4±1^{BC}	4±0^{BC}	11±2^{A}	2±0^{C}	<0.0001
Insoluble Fiber (g)	15±2^{ABC}	17±3^{ABC}	7±1^{C}	24±6^{A}	15±2^{ABC}	7±1^{C}	10±2^{BC}	419±2^{AB}	10±2^{BC}	0.0004
Pectin (g)	1.7±0.1^{AB}	5.1±1.5^{C}	1.6±0.3^{AB}	3.9±0.3^{ABC}	4.8±0.8^{BC}	1.4±0.3^{A}	4.0±0.6^{ABC}	4.1±0.9^{ABC}	1.1±0.3^{A}	0.0011
Alcohol (g)	0^{A}	0^{A}	35±4^{B}	8±8^{A}	0^{A}	27±12^{AB}	0^{A}	22±7^{AB}	27±3^{AB}	0.0002
Water (g)	3110±286^{AB}	4203±232^{A}	3601±180^{AB}	4330±1683^{A}	3234±266^{AB}	1979±292^{BC}	985±135^{C}	2294±387^{ABC}	1530±215^{BC}	0.0004

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

Table 4. SCFA concentration (µmol/g feces) during 24-hour in vitro fermentation by subject with no added fiber

Hrs	CA 1	CA 2	CA 3	CA 4	CH 1	CH 2	CH 3	JP 1	JP 2	P-Value
Acetate										
0	33.1±0.6	25.0±7.5	98.4±60.0	172.5±151.8	9.3±0.2	281.0*	8.2±0.0	12.9±4.7	11.3	0.2718
4	73.2±24.7	49.6±1.3	712.8±502.5	49.8± 2.7	11.7±0.1	297.3±177.8	63.3±2.2	22.1±2.4	16.2±0.1	0.2280
8	160.4±22.0^{A}	111.6±14.7^{A}	2111.9±479.9^{B}	92.6± 44.5^{A}	15.6±2.0^{A}	325.6±214.5^{A}	122.7±24.6^{A}	48.7±5.1^{A}	15.4±0.2^{A}	0.0003
12	198.4±89.3	155.6±62.0	1187.5±995.4	129.0± 25.0	19.9±2.7	1208.6±962.2	1467.2±632.9	191.0±73.6	22.8±1.5	0.3291
24	339.9±11.1	8255.4±3303.2	5297.9±816.1	125.9±24.2	43.4±0.3	5404.5±3534.1	2337.8±1881.5	261.1±48.2	24.4±2.5	0.0544
Propionate										
0	14.5±0.8	12.4±2.9	43.2±26.0	70.2±57.9	6.2±0.0	126.3	6.2±0.0	8.4±2.2	6.2	0.2032
4	51.6±32.3	110.9±5.1	357.9±272.6	38.4±0.7	6.2±0.0	394.1±230.0	40.1±1.8	17.6±1.7	7.0±0.8	0.2418
8	102.5±96.3	326.3±33.3	939.0±346.5	119.6±40.9	6.8±0.5	1193.6±1113.6	142.4±12.8	60.6±8.0	6.2±0.0	0.3695
12	233.1±198.8	246.6±171.8	724.3±636.9	275.6± 35.1	7.6±1.4	2839.7±2273.2	1834.5±686.3	298.1±126.5	6.9±0.6	0.3149
24	523.1±4.2	16423.0±6343.5	6631.9±674.4	243.1± 68.7	12.3±0.0	8404.4±7938.8	2903.2±2312.2	323.8±53.4	8.7±0.5	0.0871
Butyrate										
0	17.9±1.1	12.8±2.0	37.0±20.9	58.1± 45.0	8.9±0.1	77.6	9.1±0.0	9.8±1.5	8.3	0.3458
4	23.2±0.6	16.8±1.3	134.8±94.1	17.5± 1.0	9.2±1.0	36.0±16.0	8.3±0.0	10.3±0.1	10.1±0.3	0.2452
8	42.8±3.2^{A}	27.2±3.8^{A}	190.2±56.1^{B}	30.2± 12.7^{A}	9.1±0.8^{A}	33.2±16.3^{A}	17.2±1.5^{A}	13.2±1.6^{A}	10.1±0.4^{A}	0.0025
12	46.0±19.5	38.1±19.6	145.3±123.0	48.2± 11.4	11.8±0.9	289.1±253.6	153.1±69.3	53.0±17.5	11.2±0.0	0.5668
24	73.0±0.4	2237.6±881.5	1553.0±202.7	50.7± 14.1	17.8±0.0	1324.0±1209.4	602.6±458.1	77.9±11.1	12.0±0.2	0.0932
Total SCFA										
0	65.6±2.5	50.1±12.5	178.6±106.9	300.9±254.6	24.5±0.1	485.3	23.5±0.0	31.1±8.4	25.8	0.2700
4	147.8±57.7	177.3±5.0	1205.4±869.2	105.7± 2.9	27.2±1.1	727.4±423.9	111.6±4.0	50.1±4.0	33.4±0.9	0.2538
8	305.7±71.1^{A}	465.1±51.7^{A}	3241.1±882.6^{B}	242.4± 98.1^{A}	31.5±3.3^{A}	1552.5±1344.4^{AB}	282.3±38.9^{A}	122.5±14.7^{A}	31.8±0.5^{A}	0.0294
12	477.5±307.6	440.3±253.4	2057.1±1755.2	452.8± 71.5	39.3±2.2	4337.4±3488.9	3454.8±1388.6	542.1±217.6	40.9±0.8	0.3444
24	935.9±15.6	26916.1±10528.2	13482.8±1693.2	419.7±107.0	73.6±0.4	15133.0±12682.3	5843.6±4651.8	662.8±112.8	45.1±3.3	0.0770

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

*Standard error not calculated because only one replicate available for analysis

Table 5. SCFA concentration (µmol /g feces) during 24-hour in vitro fermentation by subject with glucose added

Hrs	CA 1	CA 2	CA 3	CA 4	CH 1	CH 2	CH 3	JP 1	JP 2	P-Value
Acetate										
0	35.9±3.3	26.2±0.1	88.1±40.2	1081.8±720.8	9.4±1.2	37.9±7.9	8.2±0.0	16.1±2.0	10.8±0.8	0.1247
4	81.2±46.3 ^{A}	118.1±26.5 ^{A}	2530.9±484.4 ^{C}	137.4± 15.6 ^{A}	9.4±0.6 ^{A}	6481.3±48.9 ^{B}	74.1±11.9 ^{A}	136.5±18.1 ^{A}	13.3±0.9 ^{A}	<0.0001
8	12077.4±914.5 ^{A}	3894.2±132.2 ^{AB}	6586.9±681.9 ^{AB}	87.7± 4.2 ^{B}	24.0±2.7 ^{B}	10475.6±5826.8 ^{AB}	250.4±182.5 ^{B}	405.2±7.0 ^{B}	34.8±5.6 ^{B}	0.0075
12	8299.9±835.1	12364.9±8063.8	4007.5± 245.1	4453.3±376.4	48.1±1.5	11997.5±4193.5	4981.8±841.8	4069.1±3873.4	43.2±2.2	0.1911
24	3832.3±1593.2 ^{AB}	2040.4±1146.5 ^{AB}	1249.1± 407.3 ^{AB}	1961.0± 18.9 ^{AB}	117.2±3.6 ^{B}	4269.6±891.4 ^{AB}	3867.3±624.2 ^{AB}	5532.3±1566.4 ^{A}	48.1±0.7 ^{B}	0.0207
Propionate										
0	14.0±1.0	12.2±0.1	40.6± 20.0	391.2± 262.0	6.6±0.3	19.3±1.8	6.2±0.0	9.4±0.5	7.5±0.2	0.1495
4	20.2±6.4 ^{A}	110.6±3.7 ^{A}	438.2± 79.3 ^{C}	32.2± 2.8 ^{A}	6.6±0.3 ^{A}	1216.9±20.0 ^{B}	38.7±4.7 ^{A}	22.3±3.0 ^{A}	6.2±0.0 ^{A}	<0.0001
8	2819.4±114.8 ^{AB}	5331.2±313.8 ^{A}	677.7± 51.2 ^{B}	25.0± 0.8 ^{B}	7.3±1.1 ^{B}	2845.8±2253.8 ^{AB}	145.1±100.8 ^{B}	75.7±2.1 ^{B}	7.6±0.8 ^{B}	0.0062
12	2592.1±1789.3	2404.3±882.5	1043.3± 3.6	2733.7±276.2	7.7±1.5	4714.3±1108.8	267.1±14.2	2721.6±2590.9	7.3±1.0	0.2160
24	4450.6±2208.1 ^{AB}	2180.9±742.1 ^{B}	1222.1± 4.2 ^{B}	2899.0± 56.1 ^{B}	9.4±0.2 ^{B}	2551.8±515.6 ^{B}	2450.0±164.8 ^{B}	7890.1±2250.0 ^{A}	6.2±0.0 ^{B}	0.0143
Butyrate										
0	16.9±1.1	13.1±0.2	32.0± 13.2	286.0± 182.7	9.4±0.1	14.7±0.9	11.0±1.1	10.7±0.6	8.7±0.4	0.1297
4	17.1±2.6 ^{A}	23.7±2.6 ^{A}	216.0± 38.0 ^{C}	21.7±1.4 ^{A}	8.6±0.4 ^{A}	417.8±8.9 ^{B}	11.8±3.5 ^{A}	12.4±0.6 ^{A}	8.9±0.4 ^{A}	<0.0001
8	2701.3±20.6 ^{A}	1700.7±0.6 ^{C}	256.4± 60.0 ^{B}	23.9± 2.0 ^{B}	12.2±2.2 ^{B}	578.8±328.9 ^{B}	25.3±12.8 ^{B}	30.6±5.3 ^{B}	10.4±0.5 ^{B}	<0.0001
12	4534.2±1618.6 ^{A}	1780.8±13.9 ^{AB}	2139.5± 389.4 ^{AB}	3481.0± 27.2 ^{AB}	19.4±0.3 ^{B}	1442.7±174.7 ^{AB}	267.1±14.2 ^{B}	1088.0±1029.3 ^{B}	12.0±0.0 ^{B}	0.0087
24	8923.9±4108.9	8001.0±3415.0	6400.5± 478.5	3060.4± 8.2	50.8±2.4	3123.3±834.6	259.6±18.6	10435.4±3207.8	13.9±0.1	0.2943
Total SCFA										
0	66.8±5.5	51.5±0.5	160.6± 73.1	1758.9±1147.6	25.4±1.1	71.9±10.6	25.4±1.1	36.1±3.0	27.0±1.5	0.1308
4	118.5±55.3 ^{A}	252.4±32.9 ^{A}	3185.1± 601.6 ^{C}	191.4± 19.8 ^{A}	24.6±1.3 ^{A}	8116.0±37.7 ^{B}	124.6±20.0 ^{A}	171.1±21.7 ^{A}	28.5±0.5 ^{A}	<0.0001
8	17598.1±1050.0 ^{A}	10926.1±445.4 ^{AB}	7521.0± 570.7 ^{AB}	136.6± 7.0 ^{B}	43.6±3.8 ^{B}	13900.3±8409.5 ^{AB}	420.8±296.0 ^{B}	511.5±0.5 ^{B}	52.8±6.9 ^{B}	0.0071
12	15426.2±4243.1	16549.9±7167.4	7190.2± 638.2	10668.1±625.3	75.2±2.7	18154.4±5477.0	8178.7±1065.5	7878.7±7493.6	62.4±1.2	0.0980
24	17206.8±7910.2 ^{AB}	12222.4±3819.4 ^{AB}	8871.6± 75.4 ^{AB}	7920.4± 29.0 ^{AB}	177.3±6.2 ^{B}	9944.7±2241.6 ^{AB}	6577.0±478.0 ^{AB}	23857.7±7024.2 ^{A}	68.2±0.8 ^{B}	0.0284

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

*Standard error not calculated because only one replicate available for analysis

Table 6. SCFA concentration (µmol /g feces) during 24-hour in vitro fermentation by subject with inulin added

Hrs	CA 1	CA 2	CA 3	CA 4	CH 1	CH 2	CH 3	JP 1	JP 2	P-Value
Acetate										
0	50.0±4.4 ^{A}	24.6±1.8 ^{A}	104.9±53.0 ^{A}	271.2± 68.8 ^{B}	8.2±0.0 ^{A}	29.7*	26.5±14.9 ^{A}	26.4±0.2 ^{A}	9.6±0.2 ^{A}	0.0058
4	221.5±18.9 ^{A}	111.8±21.5 ^{A}	4688.4±296.8 ^{C}	118.8± 7.7 ^{A}	10.7±0.5 ^{A}	13036.9±1666.2 ^{B}	79.2±9.3 ^{A}	197.2±47.7 ^{A}	15.8±1.3 ^{A}	<0.0001
8	15269.4±2077.2 ^{A}	3415.8±297.6 ^{C}	9866.8±1403.7 ^{B}	101.0± 0.5 ^{C}	23.1±0.8 ^{C}	13660.2 ^{A}	340.8±27.5 ^{C}	237.0±16.2 ^{C}	30.9±0.6 ^{C}	<0.0001
12	13034.2±1259.7 ^{A}	2146.9±306.2 ^{CD}	5500.1±1194.1 ^{BC}	2140.3± 2036.8 ^{CD}	33.4±4.4 ^{D}	10741.7±548.8 ^{A}	3583.0±352.6 ^{CBD}	6957.3±381.0 ^{B}	43.9±0.9 ^{D}	<0.0001
24	7989.1±426.1 ^{A}	1441.2±13.6 ^{C}	2118.6± 408.9 ^{C}	2186.8± 17.5 ^{C}	68.2 ^{C}	6199.7±405.0 ^{B}	2102.1±68.6 ^{C}	218.0±5.7 ^{C}	63.4±3.3 ^{C}	<0.0001
Propionate										
0	17.7±0.2 ^{A}	11.6±0.5 ^{A}	41.9± 21.0 ^{A}	87.6± 19.3 ^{B}	6.2±0.0 ^{A}	13.7± ^{A}	6.2±0.0 ^{A}	13.0±0.0 ^{A}	6.2±0.0 ^{A}	0.0063
4	40.0±4.1 ^{A}	104.2±2.3 ^{B}	483.6± 15.5 ^{D}	32.4± 1.1 ^{A}	6.8±0.5 ^{A}	1355.3±21.8 ^{C}	41.9±4.3 ^{A}	26.4±4.9 ^{A}	8.3±0.5 ^{A}	<0.0001
8	2861.8±433.6 ^{A}	3701.9±323.1 ^{C}	781.7± 80.3 ^{B}	29.7± 0.8 ^{B}	6.2±0.0 ^{B}	4642.5 ^{D}	282.2±13.2 ^{B}	54.7±3.1 ^{B}	8.6±0.2 ^{B}	<0.0001
12	3625.3±785.4	17581.8±14113.7	643.0± 92.2	1168.9± 1110.5	8.7±0.2	3237.1±6.2	2857.0±310.2	4174.7±445.4	7.7±1.5	0.3409
24	3395.2±164.5 ^{A}	1964.5±107.0 ^{B}	1160.8± 234.1 ^{C}	2233.7± 245.1 ^{B}	11.6 ^{D}	2377.2±201.0 ^{B}	2239.4±135.4 ^{B}	186.4±11.9 ^{D}	12.1±0.4 ^{D}	<0.0001
Butyrate										
0	24.0±0.2 ^{A}	13.3±0.3 ^{A}	39.2± 19.2 ^{A}	82.5± 19.7 ^{B}	9.1±0.8 ^{A}	13.2 ^{A}	11.5±0.3 ^{A}	13.9±0.0 ^{A}	8.3±0.0 ^{A}	0.0102
4	36.0±2.0 ^{A}	25.4±5.6 ^{A}	295.3± 22.0 ^{C}	20.6± 1.3 ^{A}	9.3±0.2 ^{A}	426.1±21.9 ^{B}	8.3±0.0 ^{A}	13.8±1.0 ^{A}	9.7±0.3 ^{A}	<0.0001
8	3842.7±443.5 ^{A}	1812.1±164.4 ^{C}	426.7± 121.7 ^{B}	31.9± 0.2 ^{B}	11.3±0.7 ^{B}	847.8 ^{B}	29.4±2.2 ^{B}	33.2±2.8 ^{B}	10.1±0.9 ^{B}	<0.0001
12	7990.8±1055.1 ^{A}	3143.9±235.6 ^{B}	1859.9± 53.8 ^{B}	2423.2± 2313.8 ^{B}	13.9±2.3 ^{B}	1802.6±202.2 ^{B}	456.7±72.0 ^{B}	3282.0±880.6 ^{B}	17.4±0.9 ^{B}	0.0035
24	4142.9±181.2 ^{A}	1952.6±282.5 ^{C}	2586.3± 424.9 ^{C}	2107.9± 152.2 ^{C}	27.9 ^{B}	2927.1±281.7 ^{C}	682.7±68.3 ^{B}	216.0±17.9 ^{B}	30.9±2.2 ^{B}	<0.0001
Total SCFA										
0	91.7±4.8 ^{A}	49.5±2.6 ^{A}	186.0± 93.2 ^{A}	441.3± 107.8 ^{B}	23.5±0.8 ^{A}	56.5 ^{A}	44.3±15.2 ^{A}	53.4±0.3 ^{A}	24.1±0.2 ^{A}	0.0063
4	297.5±24.9 ^{A}	241.5±29.4 ^{A}	5467.4± 290.2 ^{C}	171.8± 7.9 ^{A}	26.8±1.2 ^{A}	14818.3±1622.5 ^{B}	129.3±13.6 ^{A}	237.3±53.7 ^{A}	33.8±2.1 ^{A}	<0.0001
8	21973.9±2954.3 ^{A}	8929.8±785.0 ^{C}	11075.2±1605.6 ^{C}	162.6± 0.1 ^{B}	40.6±0.1 ^{B}	19150.6 ^{A}	652.4±42.8 ^{B}	324.9±22.2 ^{B}	49.6±1.8 ^{B}	<0.0001
12	24650.2±3100.2	22872.6±14043.0	8002.9±1155.7	5732.4± 5461.1	56.1±6.9	15781.4±340.4	6896.7±734.8	14414.0±1707.1	69.0±1.6	0.0585
24	15527.1±771.8 ^{A}	5358.3±376.0 ^{C}	5865.7±1067.9 ^{C}	6528.5± 110.3 ^{C}	107.8 ^{B}	11504.0±887.7 ^{D}	5024.2±135.7 ^{C}	620.3±35.4 ^{B}	106.4±5.9 ^{B}	<0.0001

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

*Standard error not calculated because only one replicate available for analysis

Table 7. SCFA concentration (µmol /g feces) during 24-hour in vitro fermentation by ethnicity with no added fiber

SCFA	Hrs	Caucasian	Chinese	Japanese	P-value
Acetate	**0**	82.3± 38.2	63.3±54.5	12.4±2.8	0.6230
	4	221.23± 143.4	124.1±72.1	19.2±2.0	0.5382
	8	619.1± 338.4	154.7±80.1	32.0±9.8	0.2838
	12	417.6± 253.3	898.5±409.7	106.9±57.1	0.2758
	24	3504.8± 1449.1	2595.2±1425.9	142.7±71.1	0.3086
Propionate	**0**	35.1± 15.0	30.3±24.0	7.7±1.5	0.6485
	4	140.7± 71.2	146.8±98.4	12.3±3.1	0.5152
	8	371.8± 145.4	447.6±372.8	33.4±16.0	0.5477
	12	369.9± 151.7	1560.6±806.7	152.5±98.7	0.1312
	24	5955.3± 2757.3	3773.3±2642.2	166.2±93.5	0.3717
Butyrate	**0**	31.5± 11.6	22.7±13.7	9.3±1.0	0.5454
	4	48.0± 26.0	17.8±7.1	10.2±0.1	0.4034
	8	72.6± 28.0	19.8±6.2	11.7±1.1	0.1291
	12	69.4± 29.1	151.3±84.7	32.1±14.0	0.3542
	24	978.6± 397.1	648.2±410.6	45.0±19.6	0.3132
Total SCFA	**0**	148.8± 64.7	116.3±92.3	29.3±5.2	0.6176
	4	409.1± 239.6	288.7±177.4	41.7±5.1	0.5366
	8	1063.6± 505.1	622.1±457.4	77.2±26.9	0.4037
	12	856.9± 429.5	2610.5±1275.6	291.5±169.8	0.1686
	24	10438.6± 4571.6	7016.7±4456.7	353.9±184.2	0.3426

Table 8. SCFA concentration (µmol /g feces) during 24-hour in vitro fermentation by ethnicity with glucose added

SCFA	Hrs	Caucasian	Chinese	Japanese	P-value
Acetate	**0**	308.0± 215.1	18.5±6.5	13.4±1.8	0.3606
	4	716.9± 406.4	2188.3±1357.7	74.9±36.3	0.2662
	8	5661.6± 1664.0	3583.4±2649.1	220.0±107.0	0.2297
	12	7281.4± 1995.7	5675.8±2455.1	2056.1±1962.4	0.3244
	24	2270.7± 522.7	2751.4±882.2	2790.2±1707.4	0.8949
Propionate	**0**	114.5± 78.3	10.7±2.8	8.4±0.6	0.3699
	4	150.3± 65.9	420.7±251.9	14.2±4.8	0.2413
	8	2213.3± 787.2	999.4±825.1	41.7±19.7	0.1927
	12	2193.4± 458.1	2550.6±915.7	1364.4±1316.4	0.6479
	24	2688.1± 626.4	1670.4±543.8	3948.2±2454.3	0.4239
Butyrate	**0**	87.0± 55.6	11.7±1.1	9.7±0.6	0.3537
	4	69.6± 32.8	146.1±86.0	10.7±1.0	0.3203
	8	1170.6± 413.1	205.4±145.5	20.5±6.2	0.0532
	12	2983.9± 520.4^{A}	576.4±281.3^{B}	550.0±522.5^{B}	0.0023
	24	6596.5± 1319.0	1144.5±662.9	5224.7±3281.1	0.0659
Total SCFA	**0**	509.5± 349.0	40.9±10.2	31.5±3.0	0.3615
	4	936.8± 504.1	2755.1±1695.4	100.0±42.1	0.2651
	8	9045.5± 2390.5	4788.2±3609.5	282.2±132.4	0.1414
	12	12458.6± 2127.0	8802.8±3606.9	3970.6±3801.3	0.2011
	24	11555.3± 2154.4	5566.3±1905.9	11963.0±7442.1	0.3607

Within a row, cells with different superscript letters are significantly different ($p<0.05$).

Table 9. SCFA concentration (μmol /g feces) during 24-hour in vitro fermentation by ethnicity with inulin added

SCFA	Hrs	Caucasian	Chinese	Japanese	P-value
Acetate	**0**	112.7± 39.8	19.8±6.7	18.0±4.9	0.0892
	4	1285.1± 745.0	4375.6±2772.6	106.5±55.9	0.2554
	8	7163.3± 2262.4	2877.6±2696.6	133.9±60.0	0.1318
	12	5705.4± 1756.4	4786.1±1998.8	3500.6±2001.8	0.7536
	24	3433.9±1006.3	3334.4±1234.2	140.7±44.7	0.1093
Propionate	**0**	39.7±12.5	7.7±1.5	9.6±2.0	0.0693
	4	165.0±70.4	468.0±280.7	17.3±5.6	0.2397
	8	1843.8±573.4	1043.9±901.8	31.7±13.4	0.2024
	12	5754.8±3744.7	2034.3±649.2	2091.2±1216.6	0.5833
	24	2188.6±311.5^{A}	1849.0±466.7^{A}	99.2±50.3^{B}	0.0036
Butyrate	**0**	39.7±11.2	10.8±0.8	11.1±1.6	0.0605
	4	94.4±44.1	147.9±88.2	11.7±1.3	0.3998
	8	1528.4±571.0	185.8±165.5	21.6±6.8	0.0726
	12	3854.4±1038.0	757.7±344.6	1649.7±1008.6	0.0575
	24	2697.4±344.4^{A}	1449.5±621.7^{AB}	123.4±53.9^{B}	0.0033
Total SCFA	**0**	192.1±63.5	38.4±8.1	38.7±8.5	0.0786
	4	1544.5±858.0	4991.5±3135.7	135.6±62.7	0.2576
	8	10535.4±3007.3	4107.3±3763.3	187.2±80.0	0.0943
	12	15314.5±4340.6	7578.1±2886.7	7241.5±4199.3	0.2858
	24	8319.9±1601.7^{A}	6632.8±2200.3^{A}	363.3±149.1^{B}	0.0229

Within a row, cells with different superscript letters are significantly different ($p<0.05$)

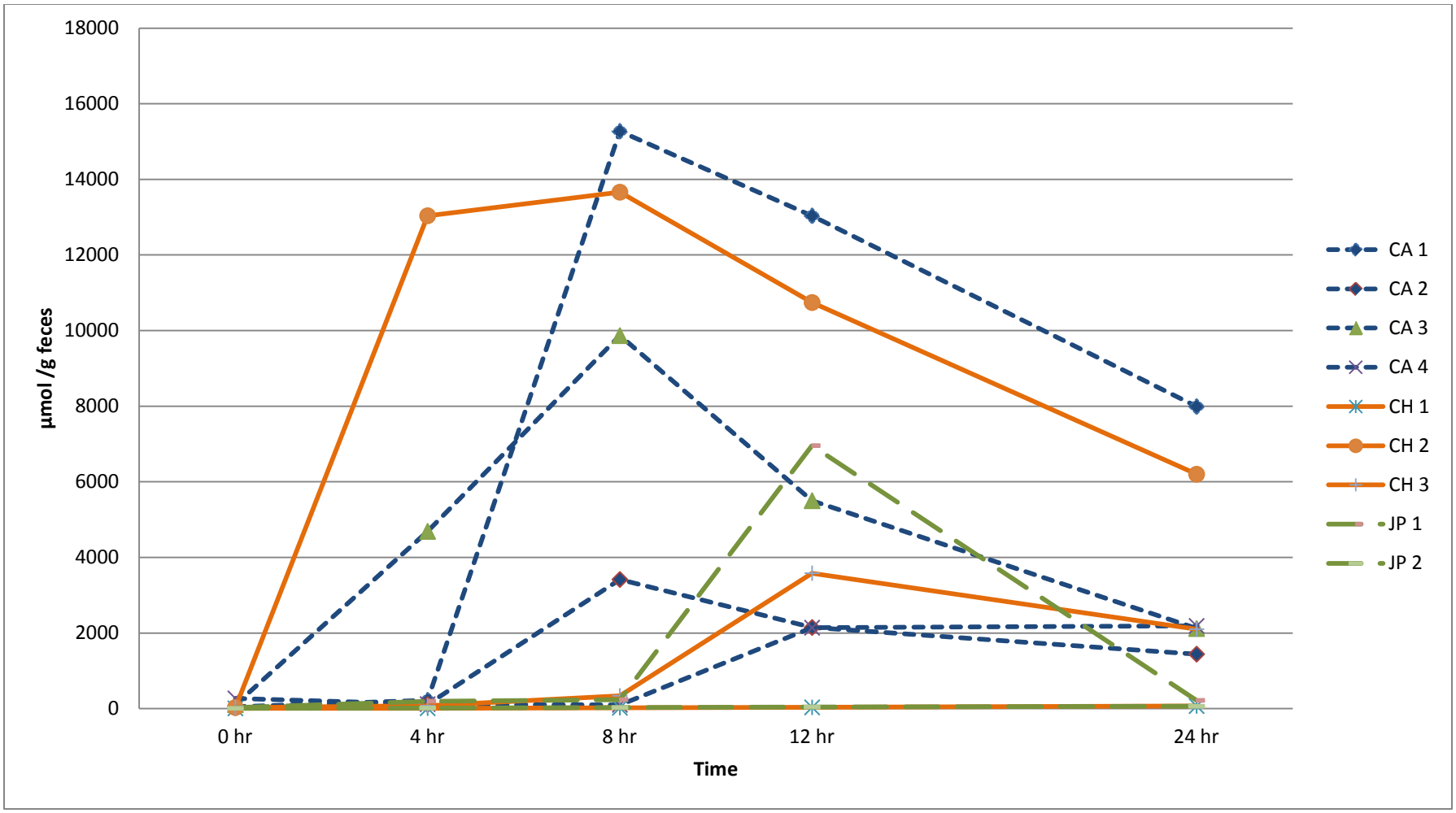

Figure 1. Acetate concentration (µmol /g feces) by subject with inulin added

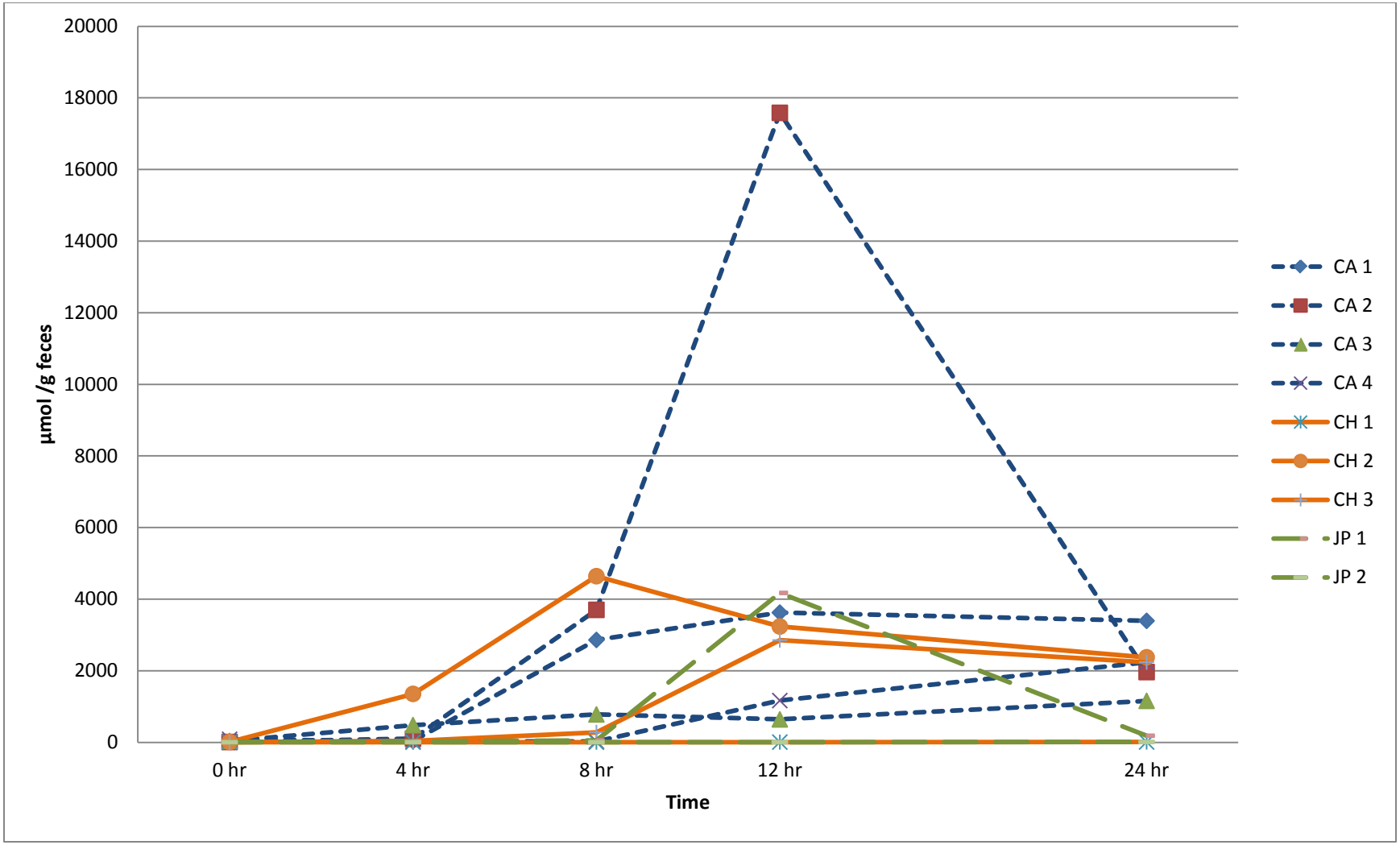

Figure 2. Propionate concentration (µmol /g feces) by subject with inulin added

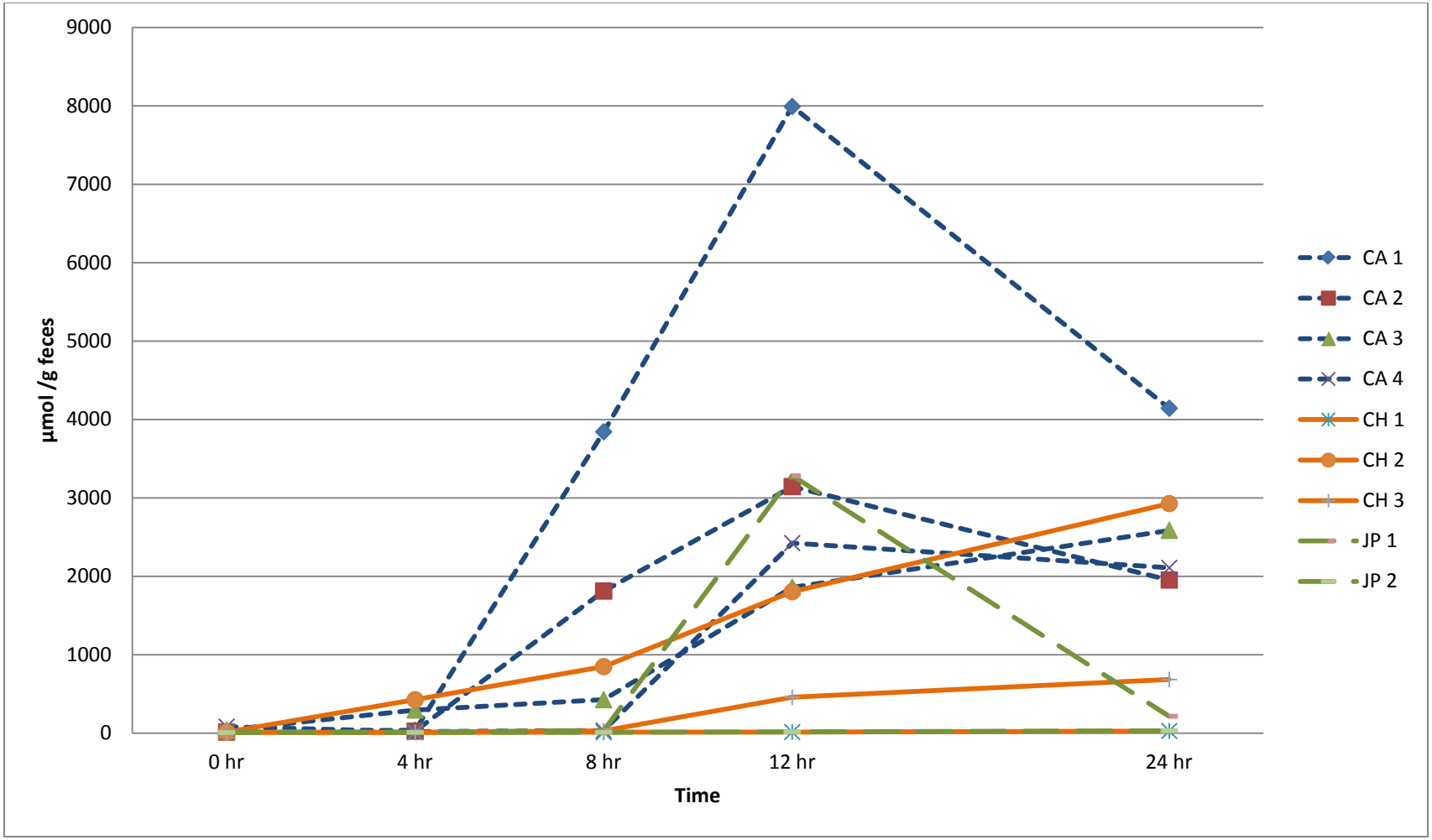

Figure 3. Butyrate concentration (µmol /g feces) by subject with inulin added

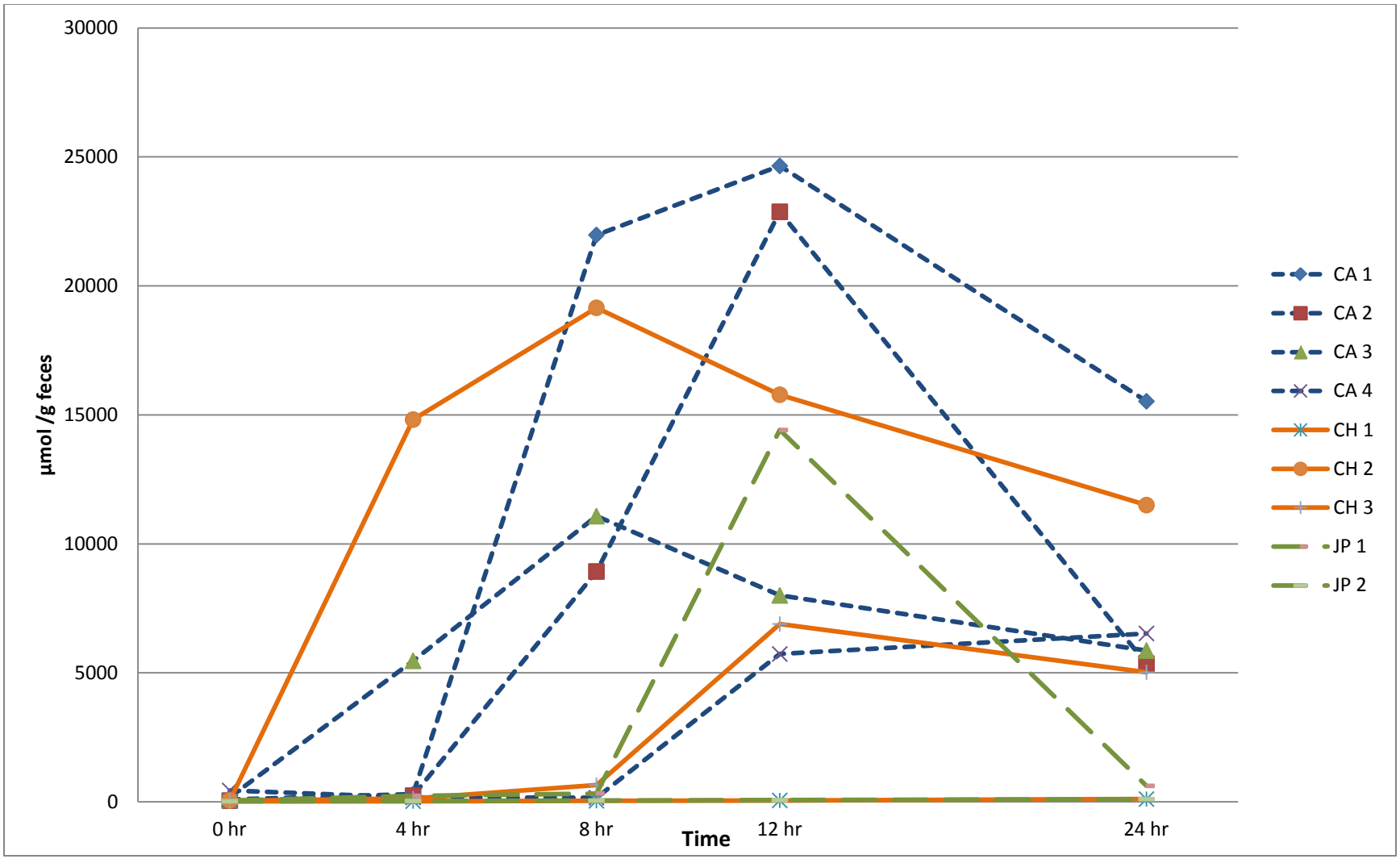

Figure 4. Total SCFA concentration (µmol /g feces) by subject with inulin added

6. References

Andrieux C, Membre JM, Cayuela C, Antoine JM. (2002). Metabolic characteristics of the faecal microflora in humans from three age groups. Scand.J.Gastroenterol. 37:792-798.

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semekovic CF, Gordon JI. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc.Nat.Acad.Sci. 101:15718-15723.

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. (2005). Host-bacterial mutualism in the human intestine. Science 307:1915-1920.

Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, Flint HJ. (2006). Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. Appl.Environ.Microbiol. 72:3593-3599.

Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. (2008). Cesarean delivery may affect the early biodiversity of intestinal bacteria. J.Nutr. 138:1796S-1800S.

Bleich S, Cutler D, Murray C, Adams A. (2008). Why is the developed world obese? Annu.Rev.Public Health 29:273-295.

Boden G, Cheung P, Stein TP, Kresge K, Mozzoli M. (2002). FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am.J.Physiol.Endocrinol.Metab. 283:E12-9.

Braun-Fahrlander C. (2003). Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002. Curr.Opin.Allergy Clin.Immunol. 3:325-329.

Brinkworth GD, Noakes M, Clifton PM, Bird AR (2009) Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. British Journal of Nutrition 101:1493–1502

Burns AJ, Rowland IR. (2000). Anti-carcinogenicity of probiotics and prebiotics. Curr.Issues Intest Microbiol. 1:13-24.

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761-1772.

Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091-1103.

Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, Dietz PM. (2007). Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care 30:2070-2076.

Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G, Stanton C, van Sinderen D, O'Connor M, Harnedy N, O'Connor K, Henry C, O'Mahony D, Fitzgerald AP, Shanahan F, Twomey C, Hill C, Ross RP, O'Toole PW. (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc.Natl.Acad.Sci.U.S.A. 108 Suppl 1:4586-4591.

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. (2012). Gut microbiota composition correlates with diet and health in the elderly. Nature 488:178-184.

Collado MC, Isolauri E, Laitinen K, Salminen S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am.J.Clin.Nutr. 88:894-899.

Collado MC, Isolauri E, Laitinen K, Salminen S. (2010). Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. Am.J.Clin.Nutr. 92:1023-1030.

Cook SI, Sellin JH. (1998). Review article: short chain fatty acids in health and disease. Aliment.Pharmacol.Ther. 12:499-507.

de Vrese M, Marteau PR. (2007). Probiotics and prebiotics: effects on diarrhea. J.Nutr. 137:803S-11S.

Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. (2005). Impact of inulin and oligofructose on gastrointestinal peptides. Br.J.Nutr. 93 Suppl 1:S157-61.

Drozdowski LA, Dixon WT, McBurney MI, Thomson AB. (2002). Short-chain fatty acids and total parenteral nutrition affect intestinal gene expression. JPEN J.Parenter.Enteral Nutr. 26:145-150.

Duncan SH, Belenguer A, Holtrop G, et al. (2007) Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and

butyrate-producing bacteria in feces. Appl Environ Microbiol 73:1073–1078.

Emma Beards, Kieran Tuohy, Glenn Gibson (2010) Bacterial, SCFA and gas profiles of a range of food ingredients following in vitro fermentation by human colonic microbiota. Anaerobe 16:420-425

FAO/WHO. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria; date accessed 11/1/13: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf

Flegal KM, Troiano RP. (2000). Changes in the distribution of body mass index of adults and children in the US population. Int.J.Obes.Relat.Metab.Disord. 24:807-818.

Fleissner CK, Huebel N, El-Bary MMA, Loh G, Klaus S, Blaut M. (2010). Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br.J.Nutr 104:919-929.

Ford ES, Giles WH, Dietz WH. (2002). Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287:356-359.

Galvez J, Rodriguez-Cabezas ME, Zarzuelo A. (2005). Effects of dietary fiber on inflammatory bowel disease. Mol.Nutr.Food Res. 49:601-608.

Gibson GR, Roberfroid MB. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J.Nutr. 125:1401-1412.

Gibson GR. (1999) Dietary modulation of the human gut microflora using prebiotics oligofructose and inulin. J Nutr 129:S1438-41.

Gourbeyre P, Denery S, Bodinier M. (2011). Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J.Leukoc.Biol. 89:685-695.

Greiner T, Backhed F. (2011). Effects of the gut microbiota on obesity and glucose homeostasis. Trends Endocrinol.Metab. 22:117-123.

Gropper SS, Smith JL, Groff JL. (2009). Advanced Nutrition and Human Metabolism, Cengage, Belmont, CA.

Guarner F, Malagelada JR. (2003). Gut flora in health and disease. Lancet 361:512-519.

Hebuterne X. (2003). Gut changes attributed to ageing: effects on intestinal microflora. Curr.Opin.Clin.Nutr.Metab.Care 6:49-54.

Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science

2001;292:1115– 8.

Huang B, Rodriguez BL, Burchfiel CM, Chyou PH, Curb JD, Yano K. (1996) Acculturation and prevalence of diabetes among Japanese-American men in Hawaii. Am J Epidemiol. 144:674–81.

John M. Eisenberg Center for Clinical Decisions and Communications Science 2007, "Childhood Obesity Prevention Programs: Comparative Effectiveness" in Comparative Effectiveness Review Summary Guides for Clinicians Rockville (MD).

Karlsson CL, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K. (2012). The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 20:2257-2261.

Labayen I, Forga L, Gonzalez A, Lenoir-Wijnkoop I, Nutr R, Martinez JA. (2001). Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. Aliment.Pharmacol.Ther. 15:543-549.

Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sorensen SJ, Hansen LH, Jakobsen M. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 5:e9085.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI. (2006). Microbial ecology: human gut microbes associated with obesity. Nature 444:1022-1023.

Lukasz Grzeskowiak, Maria Carmen Collado, Charles Mangani, Kenneth Maleta, Kirsi Laitinen, Per Ashorn, Erika Isolauri, Seppo Salminen (2012) Distinct Gut Microbiota in Southeastern African and Northern European Infants. JPGN 54: 812–816

Lupton JR, Kurtz PP. (1993). Relationship of colonic luminal short-chain fatty acids and pH to in vivo cell proliferation in rats. J.Nutr. 123:1522-1530.

Macfarlane GT, Macfarlane S. (2012). Bacteria, colonic fermentation, and gastrointestinal health. J.AOAC Int. 95:50-60.

McBurney MI, Thompson LU (1989) Effect of human fecal donor on in vitro fermentation variables. Scand J Gastroenterol 24:359-67.

McOrist AL, Abell GCJ, Cooke C, and Nyland K. (2008) Bacterial population dynamics and faecal short-chain fatty acid (SCFA) concentrations in healthy humans. Br.J.Nutr. 100:138–146

Montagne L, Pluske JR. Hampson DJ. (2003) A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108: 95–117.

Morimoto Y, Schembre SM, Steinbrecher A, Erber E, Pagano I, Grandinetti A et al.

(2011) Ethnic differences in weight gain and diabetes risk: The Multiethnic Cohort Study. Diabetes & Metabolism 37:230–236

Munukka E, Wiklund P, Pekkala S, Volgyi E, Xu L, Cheng S, Lyytikainen A, Marjomaki V, Alen M, Vaahtovuo J, Keinanen-Kiukaanniemi S, Cheng S. (2012). Women with and without metabolic disorder differ in their gut microbiota composition. Obesity (Silver Spring) 20:1082-1087.

Murphy EF, Cotter PD, Hogan A, O'Sullivan O, Joyce A, Fouhy F, Clarke SF, Marques TM, O'Toole PW, Stanton C, Quigley EM, Daly C, Ross PR, O'Doherty RM, Shanahan F. (2013). Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut 62:220-226.

National Center for Health Statistics. Summary health statistics for US adults: National Health Interview Survey (2002). Vital and health statistics, series 10, no. 222. Department of Health and Human Services publication no. 2004-1550. Washington DC: DHHS; 2004.

Nicola Abate, Manisha Chandalia (2003) The impact of ethnicity on type 2 diabetes. Journal of Diabetes and Its Complications 17:39–58

Nyangale EP, Mottram DS, Gibson GR. (2012). Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. J.Proteome Res. 11:5573-5585.

Okada H, Kuhn C, Feillet H, Bach JF. (2010). The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin.Exp.Immunol. 160:1-9.

Ollberding NJ, Nomura AM, Wilkens LR, Henderson BE, Kolonel LN (2010) Racial/ethnic differences in colorectal cancer risk: the multiethnic cohort study. Int J Cancer 129(8):1899-906

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59-65.

Ramakrishna BS, Roberts-Thomson IC, Pannall PR, Roediger WE. (1991). Impaired sulphation of phenol by the colonic mucosa in quiescent and active ulcerative colitis. Gut 32:46-49.

Roberfroid MB, Bornet F, Bouley C, Cummings JH. Colonic microflora: nutrition and

health: summary and conclusions of an International Life Sciences Institute (ILSI) (Europe)] workshop held in Barcelona, Spain. Nutr Rev 1995; 53:127–30.

Ropert A, Cherbut C, Roze C, Le Quellec A, Holst JJ, Fu-Cheng X, Bruley des Varannes S, Galmiche JP. (1996). Colonic fermentation and proximal gastric tone in humans. Gastroenterology 111:289-296.

Round JL, Mazmanian SK. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat.Rev.Immunol. 9:313-323.

Salminen S, Bouley C, Bouton-Ruault MC, et al. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998; 80 (suppl): S147–71.

Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, Sanz Y. (2010). Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br.J.Nutr. 104:83-92.

Schwierta A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring) 18:190-5

Scott KP, Martin JC, Duncan SH, Flint HJ. (2013) Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro.;FEMS Microbiol Ecol. 1111:1574-6941

Seppo Salminen (2001) Human studies on probiotics: Aspects of scientific documentation. Scandinavian Journal of Nutrition 0145:8-12

Serino M, Luche E, Gres S, Baylac A, Berge M, Cenac C, Waget A, Klopp P, Iacovoni J, Klopp C, Mariette J, Bouchez O, Lluch J, Ouarne F, Monsan P, Valet P, Roques C, Amar J, Bouloumie A, Theodorou V, Burcelin R. (2012). Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 61:543-553.

Stanley S, Wynne K, Bloom S. (2004). Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. Am.J.Physiol.Gastrointest.Liver Physiol. 286:G693-7.

Susanne Mueller, Katiana Saunier, Christiana Hanisch, Elisabeth Norin, Livia Alm et al. (2006) Differences in Fecal Microbiota in Different European Study Populations in Relation to Age, Gender, and Country: a Cross-Sectional Study. Applied and Environmental Microbiology p. 1027–1033

Suvarna VC, Boby VU. (2005) Probiotics in human health: a current assessment. Curr Sci 88:1744e5.

Tilg H, Kaser A. (2011). Gut microbiome, obesity, and metabolic dysfunction.

J.Clin.Invest. 121:2126-2132.

Topping DL & Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 81:1031–1064.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. (2009). A core gut microbiome in obese and lean twins. Nature 457:480-484.

van Passel MW, Kant R, Zoetendal EG, Plugge CM, Derrien M, Malfatti SA, Chain PS, Woyke T, Palva A, de Vos WM, Smidt H. (2011). The genome of Akkermansia muciniphila, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One 6:e16876.

Villamor E, Cnattingius S. (2006). Interpregnancy weight change and risk of adverse pregnancy outcomes: a population-based study. Lancet 368:1164-1170.

Wang Y, Wu Y, Wilson RF, Bleich S, Cheskin L, Weston C, Showell N, Fawole O, Lau B, Segal J. (2013). Childhood Obesity Prevention Programs: Comparative Effectiveness Review and Meta-Analysis. AHRQ Comparative Effectiveness Reviews.Rockville (MD): Agency for Healthcare Research and Quality (US); Jun. Report No.: 13-EHC081-EF.

Zhao G, Nyman M, Jonsson JA (2006) Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. Biomed Chromatogr 20:674-82.