

THE IMPACT OF PHYSICAL ACTIVITY AND RESISTANT STARCH
ON GUT FERMENTATION

by

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Abstract

Purpose: Physical activity (PA) and resistant starch (RS) beneficially affect metabolic health. However, their combined effects on gut health are poorly understood. The purpose of this thesis was to investigate the combined effects of PA and RS via breath hydrogen production and blood glucose responses and directly learn about the research process. **Methods:** Twenty subjects with no reported symptoms of metabolic diseases participated in this thesis project. Subjects wore accelerometers to determine PA status, and were then stratified into two groups: less active or more active. Once enrolled and stratified into groups based on PA assessment, subjects came to the laboratory on two more occasions to eat a standardized energy dense test meal with a lemonade beverage. The beverage contained different doses (5 g or 25 g) of RS type 4. On each test day, breath hydrogen was collected at baseline through the sixth hour at hour intervals through the fourth hour. Between hours four and six, the breath samples were collected every 30 minutes. Blood glucose samples were collected at baseline before the meal and then 15, 30, 45, 60, 90, and 120 minutes after beginning to eat the meal. **Results:** The incremental areas under the curve for glucose were not different between PA groups or RS dose ($p>0.05$). The area under the curve values for breath hydrogen were not different ($p>0.05$) between groups or doses of PA and RS, respectively. **Conclusion:** These results indicate that acute assessments of gut fermentation in generally healthy participants, as assessed by postprandial breath hydrogen production, requires more than six hours of assessment to determine differences between treatments and levels of physical activity.

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Dedication

I would like to dedicate this paper to my parents, Sook-young An and Jun-ok Kim.

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Chapter 1 - Literature Review

Introduction

Currently, the topic of gut microbiota is one of high interest in the health sciences. The findings of an association between chronic diseases and personalized health-care through alterations in gut microbiota have triggered many to become interested in this area (Jia, Li, Zhao, & Nicholson, 2008; Kootte et al., 2012; Neish, 2009). Gut microbiota are the clusters of bacteria colonies living in the large intestine. Gut microbiota have been found to be altered to distinctly correspond to the impact that humans encounter on a daily basis, from birth to the end of our lives (O'Toole & Claesson, 2010). This finding has helped attract scientists' attention to examine the association between diet and physical activity, with an emphasis on how they impact gut microbiota. Concurrently, several studies have reported that engaging in healthy eating and regular physical activity are associated with an alteration of the gut microbiota (Ehrenpreis, Swamy, Zaitman, & Noth, 2002; Glenn & Roberfroid, 1995).

A balanced diet (Liu, 2013) and regular physical activity (Warburton, Nicol, & Bredin, 2006) are recommended to almost all individuals due to the plethora of evidence demonstrating a positive relationship with beneficial health outcomes and ultimately decreasing the risk of numerous noncommunicable diseases (Cecchini et al., 2010). The risk of developing noncommunicable diseases, notably obesity and type 2 diabetes, can be reduced by implementing lifestyle modifications such as physical activity and healthy eating.

For example, one epidemiological study found that increasing the time spent participating in physical activity can greatly reduce the risk for several chronic diseases (Bassuk & Manson, 2005). Obesity, a specific noncommunicable disease, is associated with increased risk of

morbidity and mortality (Borrell & Samuel, 2014). The world is encountered with an increasing prevalence of obesity and in fact, it is more than doubled since 1980 to 2014 (World Health Organization (WHO, 2015). It was estimated that more than one-third of the adult population in America were considered obese (Ogden, Carroll, Kit, & Flegal, 2014). Also, type 2 diabetes mellitus threatens individuals by increasing their risk of mortality and the potential occurrence of other adverse health complications (Bassuk & Manson, 2005). A previous cohort study conducted among the U.S. population reported that noncommunicable diseases are highly interrelated. To elaborate, obesity is a predominant risk factor for developing type 2 diabetes mellitus (Ford, Williamson, & Liu, 1997). Thus, research and social interventions, such as local community health programs, need to address the increased onset and progression of several noncommunicable diseases together.

Examine an association with gut microbiota and noncommunicable diseases, gut microbiota are found to be associated with the colon environment and to the further extent, with diet and physical activity (Clarke et al., 2014; de La Serre et al., 2010; Muir et al., 1995). At the initial stages of investigating the link between gut microbiota and health, there were only a few studies published that discussed the impact of diet on gut microbiota and its composition compared with patients with a irritable bowel syndrome (IBS), Inflammatory bowel disease (IBD), and colon cancer (Ahn et al., 2013; Kassinen et al., 2007; Marchesi et al., 2007). Increased interest in gut microbiota and its potential for therapeutic treatments then began in the early years of the 21st century. Furthermore, animal studies continuously reported supporting evidence to specify an interaction in fat storage and energy metabolism with gut microbiota (Backhed et al., 2004; Ley et al., 2005). Numerous investigations initiated a search for pathological potential remedies for humans with obesity and type 2 diabetes mellitus (Kootte et

al., 2012; Musso, Gambino, & Cassader, 2011). Animal studies and clinical trials then indicated that there were differences in the bacterial population of two major phyla dwelling in the colon: *Bacteroidetes* and *Firmicutes*. These were found within a mouse model studying differences between obese and lean mice (Ley, Turnbaugh, Klein, & Gordon, 2006). With the follow-up study, among obese humans, a different proportion of *Bacteroidetes* and *Firmicutes*, independent of two different diets (carbohydrate restriction versus fat restriction diets) was found. This novel finding provided strong prospective evidence to further research in the field of obesity and gut microbiota.

In addition, the study investigated the association of physical activity and gut microbiota; this study found a higher diversity of the gut microbiota composition within more physically active group compared to those who are not (Clarke et al., 2014). Physically active individuals were professional rugby players and the control groups were recruited near the center who were male. There were total of 3 groups, rugby player, BMI lower than 25 and BMI higher than 28. The age was similar in between the groups. The finding of this study supports the hypothesis that the physical activity status would have an impact on altering gut microbiota.

In conclusion, these studies clearly mentioned that further studies are needed to understand the mechanisms behind the result of their observations. This cutting edge field of science on gut microbiota would be well-challenged in regard to aid in improving health for individuals. More importantly, current articles have not investigated human subjects' physical activity status and diet combined to examine the impact on the gut microbiota on clinical and observational studies. Therefore, it would be a novel approach to measure the impact of two components, diet and physical activity, simultaneously on the changes of composition in gut microbiome.

Gut microbiota and gut fermentation

Gut, another name for gastrointestinal tract including digestive system, is an almost exclusively system and especially its anaerobic environment provides the most adequate place for the fermentation. Gut microbiota consists of approximately 10^{13} - 10^{14} microorganisms. In addition to that, it is postulated that there are 3 million more compared to the number of our genome (Gill et al., 2006). Within the last few decades, the science in this area evolved with a tremendous input, by exploring the different species of bacteria, which are mainly dwelling in the gut. Thus, the gut emerged to the surface of current science to be investigated.

Indigestible carbohydrates, such as some dietary fibers, escape digestion in the small colon and proceed to the gut and to be the food for the bacteria residing there. The functionality of dietary fiber is to proliferate overall bacterial colonies and to benefit the environment of the large colon. The environment of gut varies due to multiple influences, thus, the gut microbiome has been found to be significantly associated with one's health and changes in lifestyle behaviors (De Filippo et al., 2010; Nicholson et al., 2012; Yatsunenko et al., 2012). This raises the possibility that the Gut microbiota might be a potential noninvasive measure for treatment or a diagnosis for noncommunicable diseases such as obesity and diabetes.

Based on previous research linking diet with chronic disease outcomes, nutrition science branched off to discover another association with diet and diversity of the gut microbiota composition (Glenn & Roberfroid, 1995). The gut microbiota could be a prospective therapeutic treatment for numerous noncommunicable diseases (Jia et al., 2008). However, the mechanisms behind the connection between gut microbiota and a predisposition to noncommunicable diseases have not been clearly elucidated.

Additionally, the anaerobic bacteria within the gut are major hydrogen producing contributors within the human body. A diet consisting of a high amount of dietary fiber may elicit a decreased amount of carbohydrate absorption compared with a diet containing a similar amount of refined and more bioavailable carbohydrate sources (Haub, Hubach, Al-Tamimi, Ornelas, & Seib, 2010). When the residual from dietary fiber traverses the colon, it will remain as "food" for anaerobic bacteria to devour (Cummings & Englyst, 1987(a)). This process occurs in the gut and called, "fermentation". It is a process that includes a conversion of a sugar to acids, and where gasses or alcohol are final products. Most importantly, it is a crucial process because those final products consist of metabolically important end-products: hydrogen, methane, carbon dioxide, short-chain fatty acids (SCFA), and biomass (Cummings & Englyst, 1987(a)). Short Chain Fatty Acid (SCFA) is found to be beneficial for colon health. The SCFA butyrate is found to be nourishing the endothelial cells in the gut, propionate is a precursor of gluconeogenesis in the liver, and acetate is used by the liver as an energy source but also used as a substrate for the synthesis of cholesterol (den Besten et al., 2013).

Previous studies have investigated SCFA pathways in vivo. It has been postulated that SCFA triggers certain gut hormones, glucagon-like peptide (GLP-1), and this hormone is considered to have anti-diabetogenic peptide effect which improves glucose and insulin control (Zander, Madsbad, Madsen, & Holst, 2002). Also, SCFA manages the control of fatty acid oxidation, thus, contributing to balance the fatty acid metabolism (Nilsson, Johansson-Boll, & Björck, 2015; Tolhurst et al., 2012). Overall, SCFA aid in weight management and lower the risk of metabolic syndrome through fatty acid metabolism, as well as glucose and insulin control.

One clinical study supported the idea that body weight impacts the composition of gut microbiota. The study examined obese, overweight and lean subjects based on BMI by

examining the amount and the compositional changes of SCFA (Schwartz et al., 2010). They examined total of 98 participants and divided them into three groups. Total SCFA was significantly higher among obese and overweight individuals compared to normal BMI individuals. However, the proportions of two major phyla: *Firmicutes* to *Bacteroidetes* was changed with the higher proportion toward *Bacteroidetes* which was contrast to the previous studies (Ley et al., 2006; Ley et al., 2005). Subsequently, it was discovered that the overall quantity of the SCFA was greater among the obese participants compare to lean participants and also the composition was different among them.

An experimental study investigated the hydrogen and serum acetate response in accordance with high and low doses of resistant starch (Muir et al., 1995). There was significantly greater production of breath hydrogen and serum acetate during high resistant starch intake compared to low resistant starch consumed. Moreover, they found that flatulence was significantly greater according to the subject's rating in high RS intake period compared with the low RS intake period. This study was assessed with breath hydrogen test. Overall, gut microbiota is a place for clusters of bacteria to ferment and metabolize indigestible carbohydrates to nourish not only the colon, but also for the overall metabolism *in vivo*.

Physical activity and health outcomes

Physical activity or "any bodily movement produced by skeletal muscles that result in energy expenditure" (WHO), is the strongest modifiable lifestyle therapy and prevention that can be applied to the greatest number of people. Currently, physical activity is deemed as a primary prevention strategy for numerous noncommunicable diseases, especially type 2 diabetes mellitus.

2008 Physical Activity Guidelines for Americans (PAGA) was the first official document published with the intent to encourage a plethora of health professionals, and scientists to utilize it as a strong resource to convey the message to public. Specifically, physical activity guidelines in 2008 recommended engaging in physical activity at the minimum of thirty minutes of moderate intensity physical activity per day for five days a week or twenty minutes of vigorous intensity physical activity per day for three days a week and including two sessions of strengthening activity a week for adult. However, increasing the time spent on physical activity for individuals is challenging even with the support of nation-wide and social programs launched throughout the United States and in each state according to the state report on physical activity, 2014.

Evidence from epidemiological studies suggests that time spent in physical activity is inversely associated with the risk of mortality, diabetes, and cardiovascular diseases (Ekelund et al., 2015). Moreover, the prospective cohort study investigated middle-aged men, who were between 45 to 84 years of age, and examined based on the physical activity questionnaires. The study found the alteration of their lifestyle factors decreased the risk of all-cause mortality (Paffenbarger Jr et al., 1993). The study included individual behavior changes (quitting cigarette smoking, maintaining normal blood pressure, and avoiding obesity) and most importantly engaging in moderate to vigorous sports activity was associated with a lower rate of mortality among middle-aged men independently. In addition, meta-analysis and reviews have shown that physical activity contributes significantly to decreased risk of noncommunicable diseases such as coronary heart diseases, stroke, and elevated biomarkers of chronic diseases (Lee, Folsom, & Blair, 2003; Warburton et al., 2006). Thus, physical activity clearly aids in the greater number of public who are at increased risk of hypertension, coronary heart disease, stroke, and diabetes.

Moore and colleagues examined a large pooled cohort of people at 40 years of age. Finding from the study suggests that those attaining the most MVPA had significantly greater life expectancy.

(Moore et al., 2012). In addition, this study found that being active and also normal weight (BMI: 18.5-24.9) increased life expectancy by 7.2 yrs compared to inactive and obese individuals (BMI>35).

Likewise, a systematic review conducted by Bize and colleagues reported an association between health-related quality of life and the physical activity (Bize, Johnson, & Plotnikoff, 2007). The assessment of health-related quality of life was attained by the SF-36 survey. In the review, most of the cross-sectional studies showed a positive association between those two variables. The cross-sectional studies that supported a positive association between quality of life and physical activity were based on data from the 2001 Behavioral Risk Factor Surveillance System survey. The result of this survey indicated that physically inactive individuals were less likely to have a high quality of life due to an increased reporting of more having unhealthy days compared to those who were physically active (Brown et al., 2003).

Physical activity, aids in attenuating a metabolic and cardiovascular risk factors (Lakka & Laaksonen, 2007). It promotes a negative energy balance by increasing the greater energy expenditure. Substantial evidence claimed that physical activity helps to control blood glucose uptake through glucose transporter protein within skeletal muscles (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974; Mul, Stanford, Hirshman, & Goodyear, 2015; Richter & Hargreaves, 2013). Therefore, problematic issues of hyperglycemia can be attenuated by improved utilization of blood glucose through engaging in physical activity. As a result, type 2 diabetes mellitus patients greatly benefit from physical activity. In addition, health advantages of physical activity include increasing insulin sensitivity and enhanced glucose uptake. Also, based on a multicultural epidemiology study, insulin played a significant role in managing type 2 diabetes

mellitus and its functionality was greatly improved by habitual, non-vigorous physical activity as well (Mayer-Davis et al., 1998). Overall, whether it is non-vigorous or vigorous, physical activity play a significant role in the glucose metabolism and control.

Accumulated evidence suggests that particularly, the moderate-intensity physical activity, such as incorporating walking into a lifestyle as a habitual physical activity, can help prevent the onset of type 2 diabetes mellitus in the general population as well as in at-risk populations (Hu et al., 1999; Laaksonen et al., 2005). The addition of regular physical activity within our daily lifestyles could become a cornerstone for treating noncommunicable diseases (NCD).

Association with non-communicable diseases (NCD) and gut microbiota

Noncommunicable diseases (NCD) also can be referred to as chronic diseases which is defined as non-infectious and non-transmittable diseases. NCD causes approximately 38 million deaths each year and approximately 28 million deaths occur within developing countries (World Health Organization, 2013). Approximately a century ago, numerous infectious diseases including influenza/pneumonia, tuberculosis, and heart diseases were the leading cause of mortality due to lack of advanced medicine and vaccines. However, current medical science has evolved to reduce those infectious diseases outbreak effectively, and the result of this brought different health related concern: chronic diseases. These diseases required a sufficient period of time to develop and become ingrained within our current society. This epidemic crisis became the biggest concern for public health. There were prominent types of NCD, those are: cardiovascular disease, cancer, respiratory diseases, and diabetes, all of which accounted for 82% of all NCD caused deaths among worldwide based on 2015 world health organization (WHO) report on noncommunicable diseases (NCD).

Type 2 diabetes mellitus

The prevalence of type 2 diabetes mellitus in the U.S. population is currently increasing as is the cost of healthcare associated with it. In 2012, the approximate cost for identified diabetes was \$245 billion and more than two-thirds of the expense was from medically related purposes, with the remaining expenses reported to be due to decreased work productivity (Centers for Disease Control and Prevention, 2014). Increasing prevalence of type 2 diabetes mellitus became burdensome to not only individual but within a family and society. Prominent long-term adverse complications caused by diabetes mellitus are cardiovascular diseases, retinopathy, nephropathy, and neuropathy which all contribute significantly to the increase of the risk of morbidity and mortality (Engelgau et al., 2004).

The onset and progress of Type 2 diabetes mellitus along with above-mentioned complications can be slowed or alleviated by appropriate physical activity (Helmrich, Ragland, Leung, & Paffenbarger Jr, 1991; Sigal, Kenny, Wasserman, Castaneda-Sceppa, & White, 2006). Epidemiological studies have shown that physical activity is essential in managing type 2 diabetes mellitus and cardiovascular diseases (Bassuk & Manson, 2005). Prospective cohort studies have reported inverse associations with physical activity and type 2 diabetes mellitus. For example, among male physicians, age and BMI-adjusted relative risk was 0.71 with 95% confidence interval of 0.56-0.91 ($p=0.006$) (Manson et al., 1992). This relative was comparing those who engage in physical activity at least once a week with those who do less than once per week as a reference. Another prospective cohort study examined among women with the follow up of 8 years. Those who engage in physical activity less than once a week was a reference compared to those who engage more than once a week. The result of this study reported that the

multivariate (adjusting for age, body mass index, family history of diabetes, and time period) relative risk was 0.83 with 95% interval of 0.74 – 0.93 ($p=0.002$) (Manson et al., 1991).

With consistent inverse associations between physical activity and type 2 diabetes mellitus, there was also another component that was added to the present study to examine the association with diabetes and gut microbiota. A recent study indicated an association between the compositional changes of gut microbiota and type 2 diabetes mellitus patients in healthy individuals at the time of initial assessments (Larsen et al., 2010). This discovery added support to the hypothesis that the presence of diabetes, including the symptoms of insulin resistance and hyperglycemia, are associated with the composition of gut microbiota.

Insulin, a significant contributor to metabolic responses, can be another factor that scientists may consider for the treatment and prevention for type 2 diabetes mellitus. Its functionality impacts blood glucose transport because it stimulates insulin-dependent glucose transporter proteins (GLUT 4), and this eventually increases the amount of glucose uptake in the blood to be stored as glycogen or used more readily to generate adenosine triphosphate via glycolysis (Richter & Hargreaves, 2013). Glycogen is a storage unit of glucose in two dominant sites in the body: liver and muscle. This is usually the form of energy that humans utilize as fuel. If the homeostasis of glucose in the blood is not regulated and insulin is no longer functioning its duty of up taking glucose which cause hyperglycemia and hyperinsulinemia status, it is called “insulin resistance” (Reaven, 1988). Continuation and exacerbation of insulin resistance will bring about cellular dysfunction. Thus, these biological events will eventually trigger the alert system in the body and this is when the predominant symptoms of type 2 diabetes mellitus, which are hunger, thirst, and fatigue, become more pronounced.

To identify whether the participants have type 2 diabetes mellitus, elevated fasting glucose is used for the diagnosis (above 126 mg/DL). The blood glucose is usually elevated among diabetes patients due to lack of transporting system within the blood to the cell. The most prominent advantage of regular physical activity is promoting insulin sensitivity which allows the insulin to work effectively in delivering blood glucose to the cell, especially to the muscle. As a result, it helps the downgraded responses of blood glucose via increases in the efficiency of insulin receptors in muscle to attenuate the insulin resistance (Goodyear & Kahn, 1998).

Obesity

Excessive body fat usually results from the imbalance between energy intake and expenditure. Obesity is typically measured using Body Mass Index (BMI), which incorporates individuals' weight (kg) and height (cm) to calculate weight in kilograms divided by the square of height in meters. A high BMI can be an indicator of high body fatness. BMI can be used to screen for weight categories that may lead to health problems but it is not diagnostic of the body fatness or health of an individual. According to World Health Organization 2015, more than two-thirds (68.8 percent) of adults (age from 20 and above) in America are considered overweight or obese (World Health Organization (WHO), 2015). Moreover, more than one-third of the United States adult population fall into the obese category in America. The National Institutes of Health (NIH) defines a BMI range from 18.5 to 25 as a normal weight, BMI range from 25 to 30 as overweight and BMI greater than 30 is obese. However, BMI has limitations in specifying the individual's location of even that there is excessive fat. Recently, determining where the excessive fat is located, has been shown to predict health outcomes more accurately as compared to simply examining individuals' BMI, specifically with excessive fat in the abdomen.

Abdominal fat can result from the excess of visceral fat. Recently this fat was pointed out to be the major contributor for the insulin resistance (Müller et al., 2012). Visceral fat is mainly located in the abdomen and its fat infiltrates into liver, this fat stored in liver is closely related with the onset of insulin resistance.

This increased body fat may ultimately cause numerous adverse health outcomes if allowed to accumulate over time (Must et al., 1999). Evidence from prospective cohort and cross-sectional studies suggest that overweight and obesity, which is measured through BMI, have a strong positive relationships with the risk of type 2 diabetes mellitus (Colditz, Willett, Rotnitzky, & Manson, 1995; Must et al., 1999). Also, previous mice studies found the connection with gut microbiota composition with the obesity. Especially, there has been successful discovering of significantly higher in total body fat composition was found within a mouse that was colonized with obese microbiota compared to control groups those colonized with lean microbiota (Backhed et al., 2004). Moreover, within human study, the scientist reported that obese and lean individuals would have different composition of gut microbiota (Turnbaugh et al., 2009a). Thus, the differences in the composition of gut microbiota influenced the body weight and possible to cause obesity.

Research has found that excessive abdominal fat and large waist circumference are risk factors for cardiovascular diseases, type 2 diabetes mellitus, and metabolic diseases (Janiszewski, Janssen, & Ross, 2007; Han, van Leer, Seidell, & Lean, 1995). A systematic review of 19 Randomized controlled trials, and 8 non-randomized controlled trials, examined the influence of physical activity on abdominal fat. The findings indicated that moderate to high intensity exercise was inversely associated with abdominal fat (Kay & Singh, 2006). Within an older

obese population, even lower intensity physical activity was found to be beneficial in reducing abdominal fat (Pescatello & Murphy, 1998). Thus, health professionals treating obese patients should promote the incorporation of physical activity within daily lifestyles.

Cardiovascular diseases

Cardiovascular Diseases (CVD) consists of numerous diseases related to vessels and blood flow connected to the heart. It includes coronary heart diseases (CHD), cerebrovascular disease, and peripheral arterial diseases (WHO, 2015). Heart disease is a leading cause of death and CHD being the most burdensome type of heart disease in America (CDC, 2015).

Approximately, 610,000 deaths are due to heart diseases each year in the United States.

Engaging in healthier lifestyles including healthy eating and adequate physical activity can prevent most of the incidence of heart disease. For instance, a study compared three levels of physical activity (low, medium and high) on life expectancy among those aged 50 years old and older with and without CVD (Franco et al., 2005). Comparing different physical activity levels and combining with life expectancy, the method utilized multistate life tables to calculate the effects of 3 levels of physical activity. Moreover, they utilized Framingham Heart Study data (DAWBER, MEADORS, & MOORE, 1951) to investigate the study. The result of this study found that those who would maintain the moderate and high level of physical activity brought 1.3 to 3.7 years more in total life expectancy and 1.1 and 3.2 more years lived without cardiovascular diseases compared to the men aged 50 years or older with maintained a low physical activity level.

Another meta-analysis of 30 studies examined the dose response of physical activity for CVD outcomes in healthy women (Oguma & Shinoda-Tagawa, 2004). They found that at least an hour of walking was associated with reduced risk of CVD among the women in the study.

Specifically, the relative risk with overall CVD and physical activity divided into 3 levels from lowest (reference) to the highest were 0.84 (0.75-0.94), 0.77 (0.69-0.87), 0.69 (0.57-0.83), and 0.67 (0.52-0.85) (p for trend 0.023). Thus, previous research suggests physical activity is a strong protective factor for risk of CVD.

Fiber and resistant starch consumption with health outcomes

Dietary fiber, an indigestible carbohydrate, structurally consists of carbohydrates that are typically resistant to absorption at intestinal tract (Eastwood & Morris, 1992). Dietary fiber is, also, one of the key intervention components for the present study. Dietary fiber has been possibly played as an unnecessary component of food for the past century where malnutrition was prevalent. However, its functionality *in vivo* brought attention to the science where the epidemic of obesity began to threaten the public health. Obesity, due predominantly to the overconsumption of food which leads to excessive energy being stored as fat can possibly aided from including dietary fiber in individuals' diet. Dietary fiber became one of potential solutions for the public health to treat obesity patients to effectively reduce their energy intake while they are satisfied with their food consumption.

Furthermore, studies began to further investigate the physiological effect of fiber *in vivo* to understand its mechanisms, and chemical structures was examined. There are two general classifications of dietary fiber based on their physical characteristics. The first category is soluble fiber, which can be dissolved in water and found widely in nature (e.g., banana, oats and many other fruits and vegetables). The second category is insoluble fiber which is not dissolvable in water and also not absorbed in our body to be utilized as energy in human body. Dietary fiber helps maintain stability of insulin and blood glucose levels (Anderson et al., 1991; Lu, Walker,

Muir, Mascara, & O'Dea, 2000). Soluble fiber attracts water and forms gel in the stomach and stays in digestive tract for a long period of time compared to simple carbohydrate (McIntosh & Miller, 2001). This delays gastric emptying and causes the prolonged absorption of glucose into small intestine and potentially provides the consumer with a longer time of feeling full. Perhaps, the most prominent advantage from dietary fiber is enhanced insulin sensitivity. This was found from feeding studies that included soluble fiber and rye bread in both animals and humans (Juntunen, Laaksonen, Poutanen, Niskanen, & Mykkanen, 2003; Song, Sawamura, Ikeda, Igawa, & Yamori, 2000). Both studies reported that diets with higher fiber content significantly increased insulin sensitivity. This finding could be contributed from better control of glucose in the small intestine, that prolonged absorption of the glucose could potentially create smooth postprandial blood glucose curve to decrease the burden for insulin to be secreted exponentially every post-meal. Another feature of soluble fiber is that it plays as a bulking agent *in vivo*, which helps to lower the serum cholesterol level by attracting cholesterol in the blood through the digestive system to the excretion.

Both types of fibers greatly impact the colon environment. Unique features of fiber also apply to resistant starch (RS), along with pectin, beta-glucan and cellulose. RS can be defined as a part of the dietary fiber group and, also, a starch. It is widely found in whole grains, bananas, legumes, and vegetables (Sajilata, Singhal, & Kulkarni, 2006). To our current knowledge, RS is broadly categorized into 4 groups from type 1 to 4 (Keenan et al., 2015). These different types of RS can be either insoluble or soluble. However, the most abundant types of RS consumed by humans would be type 3, which is a retrograded starch such as those are cooked and cooled potato or corns (Sajilata et al., 2006). It is mainly found within most human food and found within retrograded form from the heat and cool circulation. A more recent RS type would be type

4, which is chemically formulated and incorporated into most of the commercial types of resistant starch (Sajilata et al., 2006).

Previous studies have investigated the effect of RS consumption *in vivo*. A clinical study found that RS attenuated postprandial responses of plasma glucose, plasma insulin, and satiety in healthy young men (Raben et al., 1994). Another clinical study (M. Robertson, Currie, Morgan, Jewell, & Frayn, 2003) used RS (60g) with less than 2g of fiber compared among healthy individuals to measure the response of postprandial glucose and insulin sensitivity. The results showed that there was significantly higher insulin sensitivity among the RS (60g) group. Thus, we would expect that RS is beneficial for glucose and insulin metabolism. However, this study aimed to examine the short-term effect of RS intake (M. Robertson et al., 2003). Thus, they conducted the follow-up study to examine the chronic effect of RS. This study included RS (30g) for subjects compared to placebo group for 4 weeks, and results showed that the total glucose uptake by adipose tissue and insulin sensitivity was significantly greater in the RS group compared to the placebo group (M. Robertson et al., 2003; M. D. Robertson, Bickerton, Dennis, Vidal, & Frayn, 2005). Numerous studies have examined the health benefit of RS (M. Robertson et al., 2003; M. D. Robertson et al., 2005). Most prominently, satiety, glucose, and insulin biomarkers have been identified as key dependent variables following RS consumption. Unique properties of RS have intrigued scientists and many nutrition and food related health professionals. The association between the intake of RS and non-communicable diseases (NCD) needs to be further addressed.

Physical activity and gut microbiota

Physical activity is one of the highly recommended modifiable lifestyle treatments that health professionals promote for their patients with obesity and type 2 diabetes mellitus (Hill & Wyatt, 2005; Laaksonen et al., 2005). From the above information, numerous studies have pointed out the positive health outcomes related to physical activity. While physical activity is known to benefit health, scientists are inspired to further address its relationship with gut microbiota and gut health. The relationship between gut microbiota and health is a cutting edge field of science, and one of the most rapidly growing areas of nutrition research over the past two decades. Growth of this field has increased further to enhance our understanding of the association between gut microbiota and physical activity.

Regular physical activity helps to control weight, stress level, glucose control and ultimately it reduces the risk of cardiovascular diseases (Lakka & Laaksonen, 2007). Recently published studies, including animal models or human subjects, have reported the association of gut microbiota with multiple lifestyle factors, which suggests the potential possibilities of altering the gut microbiome influenced by physical activity (Petriz et al., 2014; Santacruz et al., 2009). A rat study examined the exercise induced rat those are obese, non-obese and hypertensive were compared. Within 3 groups, the study found that the within genus level, the gut microbiota was altered through 4 weeks of exercise. (Petriz et al., 2014). Another clinical study found that a relationship between physical activity and weight loss, and how physical activity could factor in to influence the alteration of gut microbiota composition (Santacruz et al., 2009). Another clinical study investigated the acute effect of exercise on the gut microbiota, and detected significantly higher production of breath hydrogen among the exercised group compared with the resting group (Ehrenpreis et al., 2002). The exercised group performed 5

minutes of running on the treadmill at 10km/h with 20% of incline. Also, this exercise was induced only within exercise trials days at 3rd hour, and consisted of a total of 420 minutes of collecting the breath hydrogen from subjects. They measured the area under the curve (AUC) from baseline to the 420 minutes, and from the 3rd hour (exercise was induced) to the 420 minute point. They observed a significant difference between the 3rd hour ($p<0.005$) compared to baseline AUC measurements ($p<0.05$).

In addition, a recent study investigated rugby players to determine whether their gut microbiome was different from those who were not as active. The professional rugby players' gut microbiome composition was more diverse compared to the control group (Clarke et al., 2014). Thus, the overall findings provide a common ground for the scientist to further investigate the connection between gut microbiota and physical activity.

Resistant starch and gut microbiota

Resistant starch and gut microbiota have both been studied to evaluate their associations with non-communicable diseases. In fact, recent studies, have already discovered that dietary factors significantly impact the composition of gut microbiota (Clarke et al., 2014; De Filippo et al., 2010; Turnbaugh et al., 2009b). Altering the diet might perhaps lead to alterations in gut microbiota composition. However, the association between gut microbiota alterations and prediction of health outcomes for individuals, is still a long way off.

Few clinical studies have investigated the alteration of gut microbiota and RS diets (Martínez, Kim, Duffy, Schlegel, & Walter, 2010; Walker et al., 2011). A clinical study compared the differential effects between RS type 2, type 4 and control treatment as native starch among ten healthy subjects. This study found that significant changes in fecal bacteria occurred

within phylum-level by significantly increasing *Antiobacteria* and *Bacteroidetes* while decreasing *Firmicutes* in RS type 4 received compared to RS type 2 and the native starch (Martínez et al., 2010). Specifically, the author of this study noted that this variation of microbiota was diverse between individuals. Thus, inter-subject variation might be more prevalent when examining the changes within their own microbiota. Following with the RS type 2 and type 4, the group of scientists examined the effect of whole-grain brown rice and whole grain barley on the gut composition. They found an alteration of gut microbiome in its two phyla *Firmicutes* and *Bacteroidetes* and an increase of its diversity (Martínez et al., 2013).

Another clinical study was conducted among overweight men to discover the effects of either RS type 3 or non-starch polysaccharide (NSP) intake on fecal bacteria (Walker et al., 2011). Specifically, there was a higher proportion of *Firmicutes* bacteria related to *Ruminococcus bromii* (*R. bromii*) detected from the RS diet compared to NSP diet. *R. bromii* is known as SCFA producing anaerobic bacteria found in human feces (Abell, Cooke, Bennett, Conlon, & McOrist, 2008). It is of note that RS diet induced the changes of fecal bacteria and possibly altered the gut composition acutely. Six out of ten male subjects' fecal microbiota's alteration was highly similar to their own previous microbiota, which represents there are higher influence from their own microbiota rather than from diet. In fact, this finding bolstered the previous clinical study (Martínez et al., 2010) with RS type 2 and 4 with the suggestion of the inter-individual differences. Thus, RS with accumulating evidence is effective in changing the gut microbiota.

While evidence exists indicating that RS intake alters the gut microbiome, there are limited data explaining the physiology and biochemistry related to how the change of gut microbiota composition directly affects the numerous chronic disease outcomes. While some

have found that SCFAs play a role in insulin sensitivity, it is less clear whether other factors (including energy balance) are as important. Thus, further clinical studies are in need to elucidate the mechanism behind gut microbiota to chronic diseases.

Study methods

Breath hydrogen test and Oro-Coecal Transit Time (OCTT) with resistant starch

An indirect and noninvasive measure of resistant starch fermentation in the gut through breath hydrogen test has practiced to help gain the knowledge on gut environment (Brewer, Weber, Haub, Cai, & Shi, 2015; van Munster et al., 1994). Likewise, previous studies have utilized the breath hydrogen test as an indirect predictor of unabsorbed carbohydrate, resistant starch and it is considered a valid tool to measure indirect quantity of gut fermentation to answer questions regarding gut microbiome. (Nilsson, Ostman, Granfeldt, & Bjorck, 2008; Rosén, Östman, & Björck, 2011). Moreover, quantifying the breath hydrogen can provide health status of the gut conveniently without having to be invasive *in vivo* (Hylla et al., 1998). The process of quantifying hydrogen begins from its production from gut fermentation. Indigestible carbohydrates such as monosaccharides and oligosaccharides travel down to the anaerobic environment, the gut. Then, glycolysis and pentose-phosphate pathway occur to break down these monosaccharides, then, are converted into pyruvates. Eventually this process produces the end products gases (hydrogen, methane, carbon dioxide) and SCFA (butyrate, propionate, acetate) (den Besten et al., 2013). These gases are either excreted through mouth, anus or utilized *in vivo*.

According to the previous study, hydrogen was produced from the intestine and 14-20% of hydrogen was reported to be detected through the breath hydrogen test (Levitt, 1969). This may degrade its applicability of measuring overall gut health; however, its noninvasive and

convenient features overcame those inaccuracies. In addition, it may remain in practice since there are limited, if any, other non-invasive alternatives. *In vivo*, there are numerous factors affecting hydrogen production. As mentioned earlier, hydrogen is consumed *in vivo* by hydrogenotroph (methanogen, sulfate-reducing bacteria, acetogen). Hydrogenotroph are the hydrogen consuming micro-organism producing methane, hydrogen sulfide, and acetate (Nakamura, Lin, McSweeney, Mackie, & Gaskins, 2010). Thus, with these numerous confounders those would naturally consume hydrogen *in vivo* would decrease the amount of detection via breath hydrogen test. There has been a suggestion that short-chain fatty acid (SCFA), end-products of anaerobic bacteria in the gut, may be one of the primary contributors in altering OCTT.

However, the breath hydrogen test is still in used and known to be an indicator for diagnosing gut bacterial overgrowth of the small bowel (SIBO). Moreover, its application has been adapted to a broader range including identifying nutrient malabsorption (ex. carbohydrate, lactose), and determining the rate of food passing through small intestine, Oro-Coecal Transit Time (OCTT) (S. V. Rana & Malik, 2014). The time when food enters the mouth to reach the small colon is called “OCTT”. There are multiple factors involved in determining one’s OCTT. However, there are two major categories: physiological factors (internal) and dietary factors (external).

Resistant starch and physical activity have an extensive impact on changing OCTT, while insulin is one of the physiological candidates for altering OCTT (Gasbarrini et al., 2009; Peters, De Vries, Vanberge-Henegouwen, & Akkermans, 2001). With normal solid meal consumption, OCTT is between 192 and 232 minutes and the duration of breath hydrogen less than 4 hours decreases breath hydrogen test sensitivity. Thus, OCTT should be considered when applying to

future research. It is noted that individuals would have different OCTT, thus, having more than 4 hours would be recommended. Also, moderate exercise was found to reduce the gut transit time and thus reducing the risk of colon cancer (Peters et al., 2001). Thus, both PA and RS intake can affect OCTT and later have impact on measuring hydrogen through breath.

Also, insulin sensitivity, how sensitive the body responds to the insulin, can play a significant role for OCTT and overall gut fermentation. A recent clinical study investigated OCTT and SIBO in type 2 diabetic patients and healthy control groups (S. Rana et al., 2016). Within type 2 diabetic patients, OCTT was significantly higher compared to the healthy control group. This result could be due to a prominent symptom of type 2 diabetes, “hyperglycemia”. It is a precursor of insulin resistance and was found to prolong duodeno-cecal transit time and ultimately reduce the rate of OCTT to have an overall effect on gastrointestinal motility (ØsterJørgensen, Qvist, Pedersen, Rasmussen, & Hovendal, 1992; Russo, Fraser, & Horowitz, 1996). Thus, it is important to consider insulin sensitivity when examining gut fermentation through measuring breath hydrogen production.

Accelerometers and moderate to vigorous physical activity

Accelerometers, devices used to measure physical activity status such as moderate to vigorous activities, have been used frequently in assessing physical activity for a free-living condition in recent years (Bassett, Troiano, McClain, & Wolff, 2015; Plasqui, Bonomi, & Westerterp, 2013). Since the mid-1990s, accelerometers have become a convenient and efficient method for objective assessment of PA (Plasqui et al., 2013; W. Robertson, Stewart-Brown, Wilcock, Oldfield, & Thorogood, 2011).

A study found that at least 7 days are required to better estimate daily physical activity of adults (Hart, Swartz, Cashin, & Strath, 2011). Another intervention study discovered approximate of 8- 9 consecutive days were required to obtain objective physical activity data with reliability of .80 for adolescents (Troost, Pate, Freedson, Sallis, & Taylor, 2000). This study also supported a 7 days-protocol for adults to wear the accelerometer to achieve valid data of their physical activity level. Accelerometers can be worn on multiple areas including the wrist, waist and ankle. Also, the type of device can determine the measuring location, which is based on whether the device is uniaxial (vertical), biaxial, or triaxial plane. Triaxial plane, theoretically, will provide the most accurate estimate and also outweigh the dominance of vertical movement of free living physical activity (W. Robertson et al., 2011). One cross-sectional study based on NHANES 2003-2004, concluded that 42% of children ages 6-11 yr are meeting the 60 mins/ day of physical activity guidelines, whereas adolescents achieved only 8%. Most importantly, only 5% of adults met physical activity guidelines (Troiano et al., 2008). This raises the awareness and the importance of physical activity among adults.

Moderate to Vigorous Physical Activity (MVPA) is considered one of the crucial factors that could help to maintain healthy body. According to 2008 PAGA, MVPA is classified as exercise intensity greater than three metabolic equivalents (MET). A MET is a metabolic unit of measure that is primarily used in clinical exercise, physiology and kinesiology science. It represents the intensity of different physical activity referenced to the resting states. One MET represents the amount of oxygen consumed while at resting status and it is approximately 3.5ml O₂ per kg body weight * min (Jette, Sidney, & Blümchen, 1990).

Moderate to vigorous physical activity time has been shown to be positively associated with positive health outcome. Previous research evidence has provided strong evidence to

support that it delays the onset of chronic diseases through improved metabolic biomarkers including postprandial glucose, lipid and insulin. Collectively, MVPA can be considered an essential activity for maintaining a healthy quality of life. To support the health benefits of MVPA, a cross cultural study found that the moderate intensity exercise decreased the fasting insulin levels with in women (Irwin et al., 2000). Also, another study investigated a 20 weeks exercise intervention among healthy participants maintaining a sedentary lifestyle (Boule et al., 2005). The subjects of this study received the exercise that progressed from 55%VO₂max for 30 minutes to 75% VO₂max for 50 minutes per sessions. Subjects received 2 sessions of exercise per week. As a result, the study discovered improved metabolic biomarkers including insulin sensitivity, glucose effectiveness and glucose disappearance index (Boule et al., 2005). Thus, the moderate to vigorous physical activity can be beneficial to our health.

However, monitoring the moderate to vigorous physical activity via accelerometer should be taken with extra care. Previous studies have found that some of the moderate intensity physical activity was not accurately measured through accelerometry such as play a golf or do house chores (Bassett et al., 2000; Hendelman, Miller, Baggett, Debold, & Freedson, 2000). These studies hypothesized that this inaccuracy of moderate physical activity is due to underestimating the upper body movement. Although, another study supported the accuracy of applying accelerometer especially, Actical to be a valid measure for the step counts of walking (Johnson et al., 2015). They found that when the subjects walked in their self-selected pace in free living situation and on a treadmill were accurate in step counts. Overall, the use of accelerometer on measuring moderate to vigorous physical activity in free living condition should be treated with more cautious manner.

Association with physical activity status and gut fermentation in young adults

Being active meaning, those individuals are meeting the 2008 PAGA (150 mins/ week for adult). Among Americans, 51.6% are meeting the guideline and those who meet both strength activity guidelines and aerobic activity is 20.6% (State Indicator report on Physical activity, 2014). Being active promotes overall health, and how that relates to the gut microbiota is an interesting connection that can be studied. Gut microbiota is found to be influenced by PA, and if that PA promotes health, there could be a potential association. In addition, RS will also, be incorporated as another independent variable to draw connections with PA. Ultimately, these two variables may have a synergistic impact on gut fermentation and gut health. Gut fermentation could potentially be altered due to the impact of PA status and RS consumption. However, numerous variables are found to be associated with the changes of gut microbiome such as insulin sensitivity, oro-coecal transit time, PH in colon, and hydrogenotroph. This line of research has not been published in the previous literature.

Conclusion

Physical activity (PA), RS and gut microbiota have been shown to impact health and are factors that promote good health. Gut microbiota, especially, emerged newly in the science in the connection with promoting health via alternating its microbiota, was found with chronic diseases such as obesity and diabetes. Physical activity and RS were found to have strong supportive evidence that they are associated with positive health outcomes. However, there are still many questions about the role of gut microbiota for long-term health outcomes and how it is concurrently affected by PA and RS intake. Particularly, the current study aims to examine whether there are effects from those two lifestyle variables on gut fermentation.

We hypothesized that PA and RS combined could enhance their potential health benefits. Likewise, the greater intensity and frequency of physical activity with increased doses of RS consumption may enhance overall health. Both aspects have been shown to independently improve metabolic health. Particularly, this study incorporates the two variables, which is considered a novel approach to examine whether there is an effect *in vivo*. Therefore, the purpose of this study was to examine the effect of physical activity and resistant starch on gut function assessed via breath hydrogen production and blood glucose responses. We hypothesized 1) the greater dose of RS will result in greater levels of hydrogen production and less of a glucose response 2) the greater dose of objectively measured PA will result in greater levels of hydrogen production and less of a glucose response.

Chapter 2 - Method

Participants

Twenty healthy-young (24.5 ± 3.4 yr) subjects were recruited within the surrounding area of Kansas State University, Manhattan, Kansas. All subjects were free from smoking, symptoms or signs of metabolic diseases such as diabetes and hypertension, and antibiotics usage for the past 3 months. They were also free from any medications relates to metabolic diseases prior to the study. Prior to participation, participants received a brief protocol description of the study with the medical history record form. Participants were screened for whether they met the physical activity guidelines or not by filling out the International physical activity questionnaire (IPAQ). If they reported exercising less than 90 minutes per week, they were assigned to

“Inactive group”, and if they did meet the physical activity guidelines, they were assigned to “Active group”. However, we also incorporated an objective measure of physical activity by requiring participants to wear an Actical for seven consecutive days. Based on their moderate to vigorous physical activity data, we regrouped and finalized the division of groups within “more active group” and “less active group”. Their weight and height were also measured on their first visit. Every component those are included in the study were reviewed and approved by the Institutional Review Board of Human Subject at Kansas State University, Manhattan, Kansas.

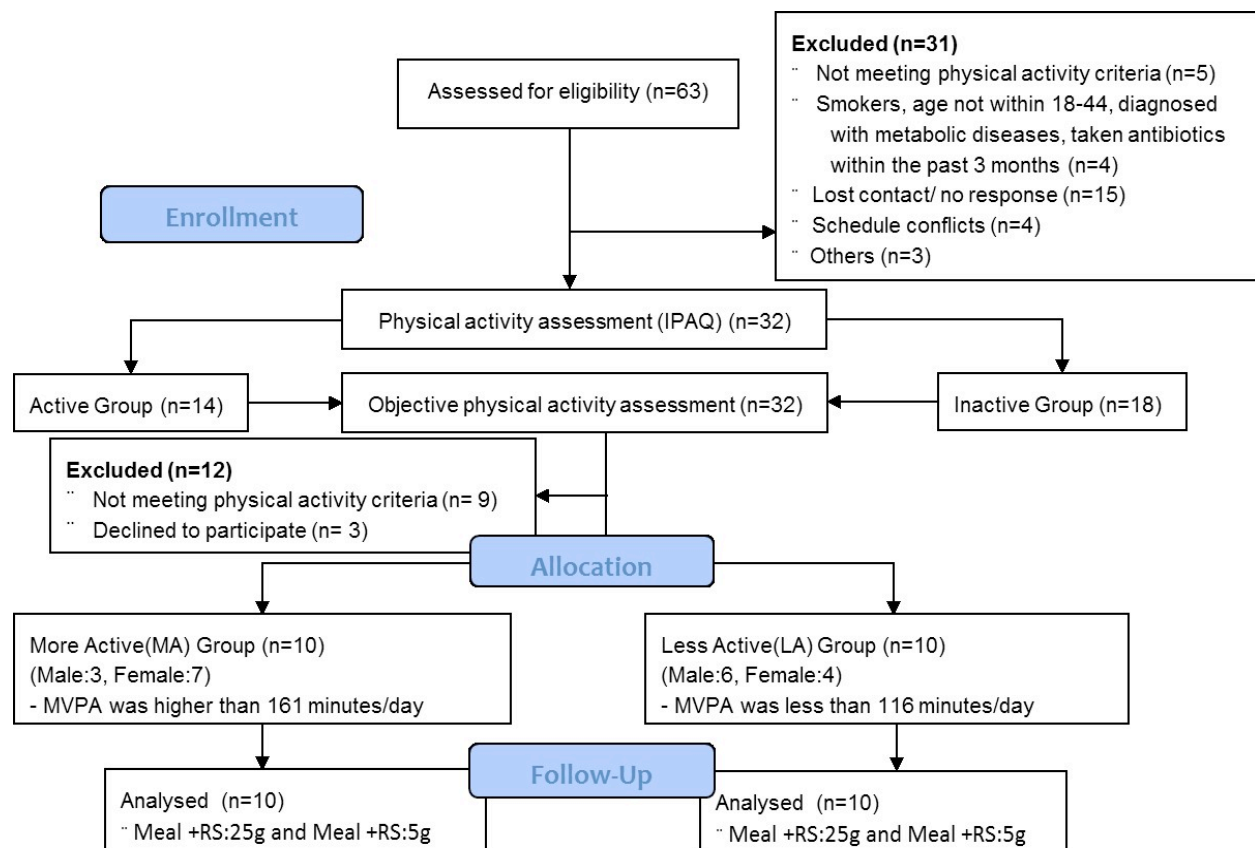


Figure 2.1 Consort Diagram for the Resistant Starch Study (RSS)

Experimental design

All subjects were required to attend a minimum of three appointments at the physical activity nutrition clinical research consortium lab (PAN-CRC). The first visit was to give the subjects a brief introduction of the study, to obtain the required information through documents

(medical history form, consent form, IPAQ, 3-day food record form) and to provide the accelerometers for wear over the next seven days. Based on the IPAQ results, participants were initially divided into two groups. A trained research assistant measured subject's height and weight and received accelerometers. Participants wore accelerometers for a minimum of seven consecutive days and then returned them to the lab with three - day food day record prior to the first feeding session. Their objective physical activity data was reevaluated to officially divide them into the active and less active groups based on Moderate to Vigorous physical activity (MVPA) minutes per day. For the total two feeding sessions, subjects consumed a breakfast bowl and lemonade on each visit with 25g and 5 g of resistant starch respectively. Meal glucose response, breath hydrogen and breath methane were measured throughout the study (approximately 6 hours at a lab). Body Mass Index (BMI) was measured through the calculation. Bioelectrical Impedance Analysis (BIA) (Biodynamics Corp.) was used to measure body composition.

Test Measurements

Pre-screener with IPAQ and Physical activity data collected through accelerometers

At the initial visit, subjects were divided into active and inactive groups based on the IPAQ responses. If their accumulated MVPA totaled less than 90 minutes, they were put into inactive group and those who were meeting the physical activity guidelines, more than 150 minutes of MVPA per week were categorized as active. However, we used IPAQ as a prescreener, and for the final determination of physical activity status, we used accelerometry (Actical, Respironics Inc., Bend, OR, USA). Subjects wore the Actical monitor on their non-dominant hand at the wrist to measure their physical activity for at least consecutive 7 days. The device was used to measure objective MVPA data to verify their self-report physical activity and

to ultimately, divide them into two separate groups. Non-dominant hand wrist was specifically chosen for convenience purpose for subjects to wear for the consecutive 7 days. A nylon band was used to secure the Actical (Accelerometer). Accelerometer was set for 30 second epochs. The light to moderate intensity cut off point was 0.031kcal/min/kg and moderate to vigorous intensity cut off point was 0.083kcal/min.kg. This was a default setting which was programmed by the manufacturer. Later, the data was downloaded and extracted into the software (Version 2.12, Respironics Inc., Bend, OR, USA).

Breath hydrogen and Methane Test

For the two feeding sessions , for the collection of breath hydrogen (H₂), methane (CH₄), and carbon dioxide (CO₂) were measured at baseline prior to the consumption of the breakfast bowl with lemonade, and every *hour* up to the 4th hour using Quintron Breath Tracker Analyzer (Quintron Instrument Company, Milwaukee, WI, USA). After the 4th hour, measurements were taken every 30 minutes. Prior to the use of the Quintron Breath Tracker analyzer, calibration took place to adjust the value of the hydrogen for the analyzer using Breath Tracker Model SC analyzer calibration gas (Quintron Instrument Company, Milwaukee, WI, USA) which contained 154 ppm of H₂, 74 ppm of CH₄, and 6.2% of CO₂. Each subject received his or her own packet with three components included for the collection of alveolar air: Mouthpiece, discard bag, and closed stopcock on a compatible syringe. Exhaled breath collection was performed according to manufacturer standards. When subjects finished exhaling into the alveosampler, then they, handed the syringe to the researcher. The researcher analyzed the collected breath by connecting the syringe to the Quintron Breath Tracking Analyzer (Quintron Instrument Company, Milwaukee, WI, USA).

Glucose Test

For each feeding session, subjects underwent a meal glucose tolerance test up to second hour. There were seven serial finger pricks that took place at baseline 00, 15, 30, 45, 60, 90, and 120 minutes after the consumption of the test meal. For each finger prick, the amount of blood drawn was less than 0.1mmol and was put on the tip of strips to put into a Bayer Contour blood glucose meter (Bayer HealthCare LLC, Mishawaka, IN, USA). Multiple measurements up to the maximum of six times were performed to ensure the reliability of the measurement. When those values were within the range of \pm six mg/dL, then, those are considered to be used for the calculation of average.

Anthropometric Measurements

Body weight and height were measured at the initial visit, and used to calculate body mass index. Subject height was measured using standard procedures with their toes together without shoes and their back straight to the measuring standard with height measured to the nearest tenth of a centimeter via a portable stadiometer (Invicta Plastics, Leicester, England). Subjects were asked to take their shoes off and weighed to the nearest tenth of a kilogram via a digital scale (Pelstar LLC, Alsip, IL, USA). Body Mass Index was calculated using the measured height and weight data using the calculation weight (kg)/height (m²), by entering the average height and weight values in the Bioelectrical Impedance Analysis device (BIA 450, Biodynamics Corporation, Shoreline, WA, USA). Body composition was also determined using BIA and was performed using manufacturer specification. Subjects were instructed to lie down in the supine position with both of their hands apart from their waist for approximately 45 degrees and the socks on their right foot were removed to show a bare skin. Two sets of signaling (red) and detecting (black) electrodes from the BIA were connected to electrode pads located on the subject's right hand and right foot. On the right hand, the signal electrode pad was attached to the

first joint of the middle finger and the detecting electrode pad was placed on top of ulnar head. On right foot, the signal electrode pad was attached to the base of the second toe and the detecting electrode was attached on top of medial malleolus. The BIA measured subject's body fat percentage and BMI indirectly through body water using prediction equation.

Meal preparation and 3-day food record

A Great Value Sausage breakfast bowl (Walmart, Bentonville, AR, USA) was cooked according to the instructions on the back of each package. The weight of food was measured with an electrical scale nearest to 0.1 g and was given to the subject based on their weight (kg). The dose provided was 10kcal of food per kg of body weight. This dose was chosen based on the previous literature to be a true to life scenario in that the meal kcal range for all subjects was 466 to 1002kcal. Minute maid premium Lemonade (The Coca-Cola Company, Atlanta, GA, USA) was served for each trial with two different amounts (5g and 25g) of resistant starch (MGP ingredients Inc., Onaga, KS, USA) added to the beverage. 250ml of lemonade was served and mixed with the different amount of resistant starch for each RS trials. Subjects were then instructed to consume all of the food and beverage provided within 20 minutes. Three-day food record data were analyzed through nutritionist pro (Axxya Systems LLC, Woodinville, WA, USA). Participants were instructed to write down the food items and portion sizes based on the instructions and example data sheet that they received. Most of the food items were consumed outside of their house such as within restaurant settings.

Statistical Analysis

Data are presented as Mean \pm SD in the tables and Mean \pm SE in figures. The sample size was determined based on the previous literature (Nilsson, Johansson-Boll, & Björck, 2015;

M. Robertson et al., 2003; van Munster et al., 1994). A Shapiro-Wilk test was performed for analyzing the normality distribution of each analyzed variable. A Mann-Whitney U test was performed for analyzing between groups differences (MA and LA) for MVPA and self-reported MVPA individually. Incremental area under the curve (iAUC) for postprandial glucose (mmol/l) and area under the curve (AUC) for breath hydrogen were measured using the trapezoid model through GraphPad prism version 6 (GraphPad software, Inc., La Jolla, CA, USA). Two-way mixed analysis of variance was performed for postprandial glucose iAUC and log transformed breath hydrogen AUC with resistant starch and physical activity as two independent factors. Two-way analysis of variances with repeated measures was performed for the postprandial glucose response, with physical activity and time as two independent factors. All the mentioned above statistics were conducted through IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA). Significant level was at $p=0.05$.

Chapter 3 - Results

Subject characteristics are presented in Table 3.1. A total of 11 females and 9 male healthy individuals completed the study. They were college students with the exception of one being a faculty member at a Midwest university. Their age, BMI (kg/m^2), weight (kg) and height (m) were measured. The age among the more active group was greater than less active group ($p < 0.05$). The other descriptive variables were not significantly different between groups.

Table 3.1 Subject characteristics

	More Active group (n=10)		Less Active group (n=10)	
Sex	Male(n=3)	Female(n=7)	Male(n=6)	Female(n=4)
	Mean \pm SD		Mean \pm SD	
Age(yr)	26.0 \pm 3.5		22.8 \pm 2.4 [^]	
BMI(kg/m^2)	23.75 \pm 3.84		25.33 \pm 4.12	
Height(m)	1.70 \pm 0.09		1.67 \pm 0.08	
Weight(kg)	69.0 \pm 16.5		71.8 \pm 17.1	
Baseline Glucose(mmol/l)	4.76 \pm 0.38		4.82 \pm 0.41	
Baseline Hydrogen(ppm)	10 \pm 13		11 \pm 11	

[^]: Less active group age is significantly different from more active group

Three-day food record and dietary summary

The three-day food record is summarized for the two groups of more and less active participants in Table 3.2. Protein, fat and fiber consumption were higher among the more active group compared to the less active group. While a higher total energy intake and carbohydrate consumption within the less active group as compared to the more active group. However, within both groups, fiber intake was lower than the recommended intake of 14g/1,000kcal. The more active group consumed 8.80g/ 1,000kcal whereas less active group consumed 6.92g/1,000kcal fiber. The more active group consumed 42% of their energy from carbohydrate and 40% from fat whereas the less active group consumed 50% of their energy from carbohydrate and 34% from fat. However, we could not find any significant difference among the groups.

Table 3.2 Three - day food record dietary summary

	More Active group(n=10)	Less Active group(n=10)
	Mean \pm SD	Mean \pm SD
Total kJ	8001 \pm 2679	8181 \pm 3482
- Kilocalorie	1912 \pm 640	2034 \pm 832
Carbohydrate (g)	202 \pm 70	252 \pm 77
g/kg b.w	3.1 \pm 1.4	3.5 \pm 0.8
% energy	44.0 \pm 11.9	51.9 \pm 7.8
Protein (g) g/kg	89 \pm 36	81 \pm 44
b.w	1.34 \pm 0.53	1.10 \pm 0.50
% energy	18.96 \pm 4.30	15.29 \pm 4.22
Fat(g)	86 \pm 53	78 \pm 46
g/kg b.w	1.26 \pm 0.71	1.05 \pm 0.48
% energy	38.06 \pm 10.28	32.30 \pm 6.05
Fiber (g) g/1,000kcal	17 \pm 10 8.80 \pm 4.15	14 \pm 10 6.92 \pm 2.97

b.w: body weight

Physical activity data

Physical activity status among groups is presented in table 3.3. Steps, objectively measured MVPA, and self-reported MVPA were analyzed between the more and less active groups. Steps between two groups were significantly different. The more active group showed higher steps (19728) compared to less active group (11486) ($p<0.05$). The objectively measured moderate to vigorous physical activity (MVPA) per minutes per day was analyzed. Actical accelerometers, were returned in good condition; and, overall, the participants complied well. When the division of physical activity occurred in accordance with the objectively measured MVPA, 6 people were moved to the more active group even though they did not meet the physical activity guidelines and two others were moved to less active group. The dividing line was drawn between less than 116 minutes per day of MVPA and more than 160 minutes per day of MVPA. Both of the groups exceeded the recommendation of physical activity guidelines (physical activity guideline 2008). The more active group obtained significantly more MVPA compared to less active group. ($p=0.000$). Also, we calculated each participant's mean MVPA (min/day) into minutes per week to further visualize the difference from self-reported MVPA data. However, within self-reported MVPA, there was no significant difference among the groups. These groups' individuals were later put into more active and less active regardless of their answering to the self-reported MVPA. However, it is important to note that both of the groups met the physical activity guidelines when it was calculated as MVPA minutes per week. This phenomenon will be further discussed in the following chapter.

Table 3.3 Physical activity summary

	More Active group(n=10)	Less Active group(n=10)
	Mean \pm SD	Mean \pm SD
Steps (day)	19728 \pm 5256	11486 \pm 3997 [^]
MVPA (mins/day)	204.2 \pm 41.2	95.8 \pm 24.1 [^]
(mins/week)	1429.4 \pm 288.5	670.6 \pm 168.9
MVPA(self-report) (mins/week)	141.5 \pm 173.6	109.5 \pm 125

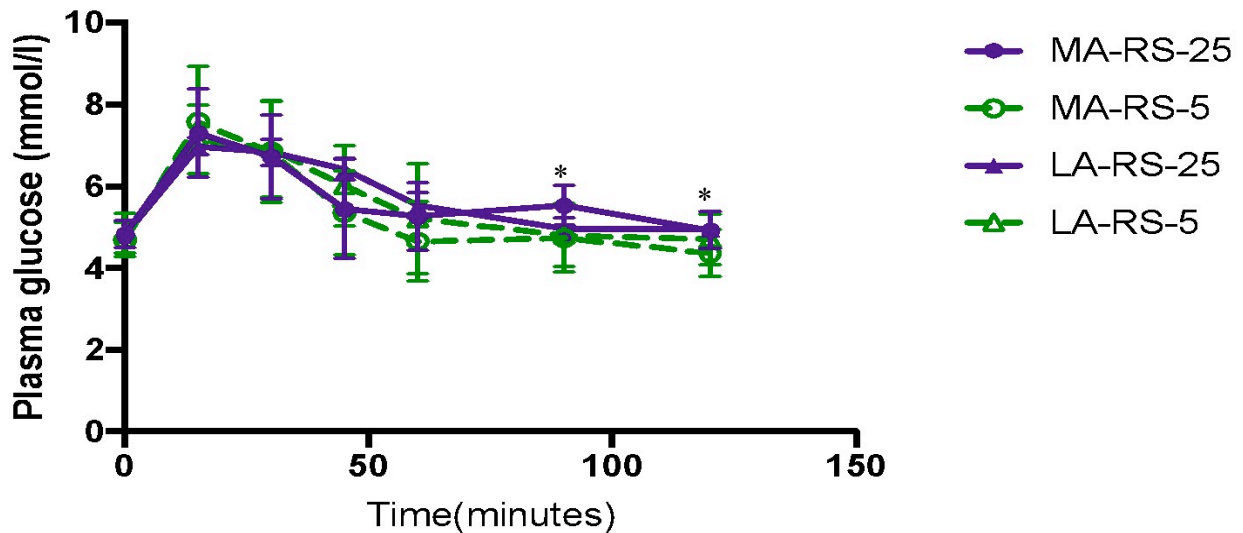
[^]: Less active group is significantly different from more active group ($p<0.05$)

Postprandial glucose responses

Postprandial glucose response for 120 minutes was measured following two feeding sessions of the standardized energy dense meal with 25g or 5g of resistant starch added to standard sweetened lemonade, results displayed in figure 3.1. Each of the four groups are represented as more active (MA), less active (LA) with 25g or 5g of resistant starch consumed. Individually, MA with 25g (MA+25) reached the highest at 15 minutes and its response increased at 90 minutes and decreased at 120 minutes. MA with 5g (MA+5) glucose levels also showed the increase at 90 minutes and decreased at 120 minutes. Among MA, the absolute change (A value – A's previous value) were highest at the change to 45 minutes (1.28, 1.50 mmol/L) compared to the rest of absolute change in glucose response. Also, at 90 and 120 minutes, there was a statistically significant difference between the 25g and 5g conditions within MA where the 25g condition demonstrated a higher glucose value as compared to the 5g condition. The LA with 25g

(LA+25) reached the highest response at 15 minutes and then continued to decrease. The absolute change increased at 45 minutes and 60 minutes (0.61, 0.77, and 0.91). LA with 5g (LA+5) also showed the highest response at 15 minutes and then continued to decrease. Among LA, there was no increase in glucose response at 90minutes.

Figure 3.1 Postprandial glucose response for 120 minutes



Mean \pm SE, * represents significance at $p < 0.05$.

*MA with 25g intake significantly different from MA with 5g intake ($p < 0.05$)

Glucose response incremental area under the curve (iAUC)

Postprandial glucose response incremental area under the curve (iAUC) among the four groups is represented in table 3.4. The MA+25 group experienced the lowest value among the groups, but was not significantly different. MA+5 demonstrated the second highest iAUC value among the groups for glucose. Also, LA+25 showed the highest iAUC out of rest of the group. LA+5 showed the second highest which was higher than MA+25. Overall, there was no significant difference found within interaction and main effect from RS and PA (P).

Table 3.4 Incremental area under the curve (iAUC) for postprandial glucose for all groups

	More Active group (n=10)		Less Active group (n=10)		$RS \times P^{\&}$	$RS^{\&}$	$P^{\&}$
	RS 25	RS 5	RS 25	RS 5			
iAUC glucose*	118.2±52.7	125.6±40.7	126.6±45.3	122.6±62.7	0.659	0.894	0.891

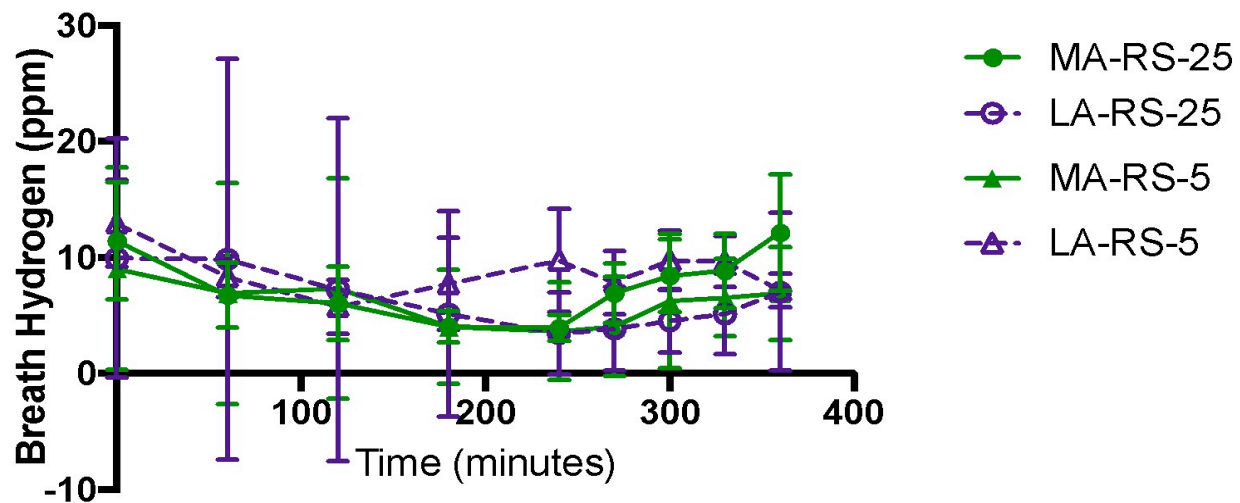
Mean ± S.D. *: Mmol/hr/L

&: indicates two way mixed analysis of variance was conducted: interaction effect of RS and PA, main effect from RS and P, *p*-value.

Postprandial breath hydrogen responses

Postprandial breath hydrogen (ppm) throughout the six-hour assessment for the more and less active groups are displayed in figure 3.2. MA+25 showed the lowest response (3.9 ppm) at 240 minutes and then, raised up to the highest at the 360 minutes. Also it was the only group that exceeded the baseline breath hydrogen compared to the 360 minutes response (11.4 vs 12.1). MA+5, also reached the lowest point at 240 minutes within its responses, and increased until 6th hours but did not exceeded the baseline breath hydrogen (9.0 vs 6.9). Also, LA+25 represented the identical trend that at 240 minutes, the response was the lowest and steadily increased (9.9 vs 7.0). Overall, the three groups except for LA+5 reached the lowest response at 240 minutes and increased until the 6th hour. However, LA+5 reached its lowest hydrogen response at 120 minutes and increased and decreased thereafter until 6th hours. The hydrogen response in LA+5 remained high throughout the up and downs (7.1- 9.7). . The last group, LA+5 showed its unique trend of rising at second hour and fluctuated throughout the rest of 4 hours. However, there were no significant differences between the groups.

Figure 3.2 Postprandial breath hydrogen for 360 minutes



Mean \pm S.E.

Breath hydrogen response area under the curve (AUC)

Postprandial breath hydrogen area under the curve (AUC) was displayed in table 3.5.

There was no significant interaction effect and main effects with physical activity (PA) and resistant starch (RS) dosage ($p>0.05$). MA+25 represented as the second highest hydrogen AUC among the groups. MA+5 demonstrated the lowest hydrogen AUC among the groups and this can be due to the differences in the dosage of RS. However, LA+25 represented the second lowest hydrogen AUC. Moreover, LA+5 had the highest hydrogen AUC, whereas, MA+5 had the lowest hydrogen AUC. Among the more active group, RS 25g consumed was greater compared to the RS 5g consumed. This result coincides with our first hypothesis partially. However, among the less active group, RS 5g consumed produced greater breath hydrogen compared to the RS 25g consumed. This inverse result brought us to answer further explanation.

Moreover, we included the calculation from fourth hour to the sixth hour AUC of breath hydrogen, which is displayed in table 3.5. It is important to understand that the certain time is required for fermentation. A study discovered that at least 90 minutes are needed for the food to reach to the

cecum and this time can be extended or truncated due to other food items consumed together to produce hydrogen from fermentation (Ghoshal, 2011; Tadesse, Smith, & Eastwod, 1980). Thus, the fourth hour AUC breath hydrogen calculation was used to capture hydrogen produced from the fermentation of the meal consumed.

Table 3.5 Postprandial breath hydrogen's area under the curve (AUC), log transformed AUC and from 4th hour AUC

	More Active group (n=10)		Less Active group (n=10)		$RS \times P^{\&}$	$RS^{\&}$	$P^{\&}$
	RS 25	RS 5	RS 25	RS 5			
AUC hydrogen	2424±2100	2129±2065	2283±3110	3045±2962			
AUC Hydrogen [^]	3.2±0.5	3.2±0.4	3.2± 0.4	3.3±0.4	0.369	0.415	0.622
AUC hydrogen [#]	963±1096	659±462	558±388	1065±870			

Mean ± S.D. All of the variables were calculated with ppm * minutes.

[^]:Log10 transformed data were used.

[#]: 4th hour calculation on AUC hydrogen

[&]: indicates two way mixed analysis of variance was conducted: interaction effect of RS and PA, main effect from RS and P, *p*-value.

Chapter 4 - Discussion

Major findings

Our study did not support our hypotheses, which were to find the greater production of breath hydrogen production within the greater dose of resistant starch nor the greater levels of objectively measured physical activity. Also within glucose response, we did not satisfy our hypotheses. However, it was observed that there were no significant differences among the groups including MA with 25g and 5g and LA with 25g and 5g. The interaction effect and main effects were not significantly different. These findings may be in contrast to previous studies that reported an increase in breath hydrogen production with higher resistant starch received (van Munster et al., 1994). In one study, subjects received a total of 28g of resistant starch per day within 7 consecutive days which can be considered an abnormally high amount of intake per day. Our study, however, provided 30g of RS in one serving in one day in order to examine the acute effect of a high dosage of RS. These different study protocols might have caused these disparate results. However, consuming 27g or resistant starch for every day for seven days within a population with normal diet pattern, could have led to difficulty adjusting to the new diet. In fact, during 1999-2002 in the U.S., approximately 4.9g of resistant starch was consumed on a daily basis in people over the age of one (Murphy, Douglass, & Birkett, 2008). The range of this estimation was from 2.8 to 7.9g per day and most of the resistant starch was consumed in food items such as bread, cooked cereal, pastas and vegetables, not including legumes (Murphy et al., 2008).

Also, another possibility that can explain not finding the significant difference from our study is that we incorporated RS in a liquid, lemonade. This is different from previous studies where others provided the RS mixed within bars, syrups or muffins (Al-Tamimi, Seib, Snyder, &

Haub, 2010; Jenkins et al., 1998; Raben et al., 1994). What is different from other studies is that the food was a typical western meal that could be easily found within daily life. On top of this, it utilized a traditional and easily accessible meal menu by including lemonade, which can be reproduced by others. The sausage breakfast bowl with the lemonade was provided for the subjects. However, we employed a more true-to-life meal using more realistic meals as compared to previous studies. We also did not find any significant difference in glucose and hydrogen response from consuming this meal. In other words, the meal was tolerable among healthy individuals and did not elicit higher or lower glucose or hydrogen responses. It is easy for scientists to view as reductionist when it comes down to analyzing whether A effect B. However, true to life scenario, is not something we can restrain all the confounders to exactly examine the direct relationship with A and B. Thus, with our study focusing true to life scenario utilized the most easily attainable products for the subjects.

We provided the energy dense-typical westernized meal including a high amount of fat. We provided the sausage breakfast bowl containing 58% of fat along with RS contained 250 ml of lemonade and postulated the mixture of macronutrients, especially high in fat would affect the metabolic response. The high-fat meal composition may have explained the lack of significant differences in breath hydrogen production. Another potential explanation is that there might be an adaptation effect to this high-fat meal for each subject *in vivo*. Thus, we collected the three-day food record to examine their typical meal pattern and the day before the actual trials.

We found the macronutrient intake among the groups were not statistically different. Based on the three-day food record, the LA group fiber intake was lower compared to MA. Also, macronutrient composition such as carbohydrate (g) intake was higher among the LA group compared to MA group whereas the fiber intake was lower. However, the fat intake was slightly

higher among the MA group. Thus, overall kilocalorie was not different between the LA and MA groups. There may have been a simple carbohydrate and complex carbohydrate consumption difference. However, we were unable to analyze the data with simple and complex carbohydrate due to lack of information provided from the three-day food record. Numerous sources were included within the category of carbohydrate, from simple crackers to the whole-wheat buns which could have caused the physiological difference in glucose response, insulin response and possibly with these cumulative influences from diet might have caused individuals to have different transit time in digestive system. According to the three-day food record, two subjects consumed alcoholic beverages on the day before the trials. However, their baseline breath hydrogen response was not much different from others.

In conclusion, we did not detect an impact for physical activity status on hydrogen production following consumption of RS. This could be due to other possible confounders such as not diverse enough among the groups' chronic PA status, age difference, and carbohydrate and fiber consumption difference among the groups. LA had more subjects with higher BMI, and also LA consumed more carbohydrates and less fiber, protein and fat. These variables could potent in a highest LA+5 hydrogen production. However, the result of hydrogen production still remained as a question and a controversial topic *in vivo*. Thus, this discussion will further elaborate on the features of resistant starch type 4 as dietary factor and physiological factors those may involve with the impact of overall physical activity and resistant starch.

Impact of resistant starch type 4

Our group previously reported that there was a protective effect on postprandial glucose and insulin from consuming resistant starch type 4 (Al-Tamimi et al., 2010). In the previous study, our group reported that resistant starch type 4 is beneficial for glucose response and insulin

sensitivity. There was significant difference compared to glucose response and insulin secretion which represented its benefit with RS type 4 in wheat starch compared to identical available carbohydrate (Al-Tamimi et al., 2010). Within our present study, we used resistant starch type 4 which contains more dietary fiber and resistant starch component compared to RS type 2 (Haub et al., 2010). The previous study which examined study found that RS type 4 elicited significantly lower glucose compared to RS type 2. This allowed us to support that it is more beneficial for health compared to RS type 2 (Haub et al., 2010). Resistant starch can perhaps ameliorate the spike of glucose within healthy individuals and also benefit the glucose metabolism.

RS type 4 is comparatively new in the field of resistant starch and known for its health benefit. However, our study did not detect significant increase in breath hydrogen but, among postprandial glucose response, there was a sustained glucose effect among MA+ 25 at 90 and 120 minutes compared to MA+5. Within our study, there was also no complaint from the discomfort from flatulence or any other symptoms from stomach among subjects. This could provide data for further studies examining dose response of resistant starch type 4 within healthy individuals. However, there were a few complaints related to the taste and appearance of the lemonade. The lemonade was mixed with the resistant starch type 4, thus, the color of lemonade was opaque and taste may have been little different from original. Overall, administering resistant starch type 4 into a liquid form can be considered adequate to elicit the health benefit specific to glucose metabolism.

Limitation in physical activity status measure

Physical activity was assessed via accelerometers placed on each subject's wrist. This increased the compliance with all of the subjects. However, possible limitation of the objective measure of physical activity should be taken into account. The results from objectively measured

data showed that both groups met the recommendation of 10,000 steps per day and PAGA recommendation of 150 minutes per week of MVPA. This raised some questions regarding their daily lifestyles. Most of the subjects who were moved into the more active group were usually occupied with work which requires moderate to high physical labor at food service. Practically, the subjects were all college-aged students (except for one instructor) who would walk to classes most of the time and walk back to their houses or dorms. There were two people who lived in the dorms, two who commuted either by walking or biking and lived near campus, and the rest were graduate students among the less active group. Despite the fact that they were put into the less active group, they were still meeting the PAGA. Suggesting the wrist based accelerometers may overestimate MVPA in this population, or perhaps our sample was simply more active than national PA statistics suggest. Perhaps, our recruitment was simply not successful in gathering inactive of our population. Also, six people who considered themselves as inactive were turned out to be a lot more active than they assumed, and were put into the more active group.

In addition to the limitation of physical activity status measure, we did not find the significant difference among the two groups with breath hydrogen. The reasoning for this might be due to the inadequate diverse physical activity status among the two groups. Both of the groups, in fact, met the PAGA. I would just suggest that there might not be group differences as we were not capturing those who were truly inactive. Perhaps just not enough difference between groups.

Determinants of physiological variation

Habitual physical activity and the combination of resistant starch type 4 may not be able to explain for this result of not finding any significant difference due to the fact that it might be more complex than what we originally hypothesized. We hypothesized that the more active individuals or the high dosage of RS would elicit more hydrogen production compared to the less

active or the low dosage of RS. However, our hypotheses were not supported. Likewise, within the 40 total trials, we observed high variability among subjects' breath hydrogen production, which ranged from 0 to 50 ppm at baseline and ranged from 2 to 58 ppm at the peak. This high variability may have resulted from inter-individual differences including physiological influences such as insulin sensitivity, hormones (which are involved in managing the absorption of carbohydrate), OCTT, PH and the site of the colon, and BMI. All of these factors are highly interrelated with regard to noncommunicable diseases along with gut health.

In a previous study, insulin sensitivity was examined due to the possibility of it play an influential factor in hydrogen production among healthy individuals, however, the lack of evidence brought inconclusive result for its relationship (Robertson 2005). Also, there are few studies examining the relationship between insulin sensitivity and hydrogen production. One of the studies examined short chain fatty acid (SCFA) production and insulin sensitivity (M. D. Robertson et al., 2005). SCFA is produced along with hydrogen from gut fermentation, thus, we could indirectly measure the benefit of the SCFA through hydrogen production. It compared the metabolic response including SCFA (butyrate, acetate, and propionate) and insulin sensitivity. During meal tolerance tests (MTT), the RS supplementation group's insulin sensitivity was higher by 33% and SCFA, acetate and propionate concentrations were significantly higher compared to placebo group as well. Based on previous studies, we would like to explore SCFA more in depth to narrow down the possible reason for not finding the effect from RS and PA within our current study.

Previous studies have connected the dots between metabolic response and SCFA to determine the association. Another finding from the above mentioned clinical study investigating SCFA role *in vivo* (M. D. Robertson et al., 2005) was that there were significantly higher

concentrations of acetate and propionate and also in acetate uptake by adipose tissue and muscle tissue compared to placebo group ($p=0.01$). Of note, RS provided the potentially higher amount of indigestible carbohydrate to feed the anaerobic bacteria in the colon. Then, this led the enrichment of the tissues not only within the gut but also within adipose and muscle tissue. This could potentially correlate with a better control of insulin secretion and insulin sensitivity. Thus, it reaffirmed the potential health benefit of producing the end-products, SCFA. Recently, a human clinical study conducted in Europe found that peripheral insulin sensitivity was significantly increased within a group of obese males due to the fecal transplant from lean fecal donors (Vrieze et al., 2012). Also, the finding of increased diversity of gut microbiota was captured. This evidence aided in the support of the importance of gut microbiota diversity. Especially butyrate related gut microbiota showed a 2.5-fold increase in fecal sample among allocation group, which is the group that received fecal transplant from lean donors. Obese group originally classified with lower microbiota diversity, however, the gut microbiota diversity was significantly increased after the infusion of lean donor's fecal transplant. Among those received fecal transplant from lean donors showed that sixteen bacterial groups significantly increased, including a subset of six other bacterial groups were significantly different from obese group before the fecal transplant. Thus, fecal transplant showed that the alteration of gut microbiota is possible from those who are lean to benefit insulin sensitivity.

Another important physiological determinant would be the hormones involved in the metabolism of carbohydrate. Insulin, which we have discussed above is the most prominent hormone involved in this process. However, there are others important hormones involved in this process such as ghrelin, and glucagon-like peptide-1 (GLP-1). A clinical study mentioned above that provided 30g of resistant starch type 2, also investigated ghrelin, and discovered an increased

fasting ghrelin concentration compared to placebo group (M. D. Robertson et al., 2005). The placebo group received 20g of Amioca (type of starch) which includes 20g of rapidly digestible starch. This finding raised few questions based on the role of ghrelin *in vivo*. Ghrelin is known to trigger hunger. However, plasma insulin AUC was lower among the high-fiber group compared to placebo. Also, by examining the postprandial ghrelin response, the baseline of the RS supplementation group began at the higher point compared to the placebo group. This may be the cause of significant difference among the groups. Upregulated ghrelin and SCFA might be highly associated with the elevated insulin sensitivity from RS supplementation. Moreover, GLP-1, part of incretins and known to stimulate insulin release, did not have an effect from RS supplementation.

Differing from other clinical studies, there was one study which examined the fecal SCFA including butyrate, acetate, and propionate with RS type 2 (21.5g of RS), RS type 3 (27.9g of RS), control (low fiber, 2.3g of RS) and wheat bran (1.5g of RS) per 2 weeks for each treatments (Jenkins et al., 1998). The study showed significantly higher mean butyrate:SCFA ratio along with the increased fecal bulk among the high RS diet group. The reduction in the fecal butyrate:SCFA ratio is commonly found within colon cancer patients (Weaver, Krause, Miller, & Wolin, 1988). Thus, this increased ratio of butyrate: SCFA could indicate the beneficial effect of high resistant starch diet on colon health. Moreover, butyrate is consumed by colonocytes as a nutrient. This study also examined the breath hydrogen production within the four groups. The study reported that there was no significant difference within four groups. The author pointed out the reason for this phenomenon was due to the slow rate of fermentation that they could not detect the hydrogen production via breath hydrogen test within 12 hours. Likewise,

our study also could not detect the difference among high and low RS conditions or PA status groups.

Another clinical study found that a significantly higher production of hydrogen was detected between a high resistant starch (59.1g consumed per day) compared to low RS diet (5.2g per day) (Muir et al., 1995). However, the measure of hydrogen production was over 28 hours and this could have contributed significantly in the detection of different hydrogen production among low and high RS groups. Their rise occurred at 20-22 hour, which is 8-10 hours after they started to collect the breath hydrogen. Also, the previously mentioned study with the finding of a higher butyrate:SCFA ratio, could not detect the higher hydrogen production whereas with this study analyzed the hydrogen up to 28 hours, allowed detection of the higher production of hydrogen with high intake of RS. Thus, this timing such as with OCTT with each individual could played significant role in affecting the whole breath hydrogen production outcome. Attaching this knowledge to our study, if we extended the time period per trial, we might have seen higher production of breath hydrogen among MA+25. Specifically, this group showed the highest absolute change among the four groups at the last time interval from 330 minutes to the 360 minutes (5.5, 1.8, 2.7, and 3.5). We postulated this observation was due to higher intake of RS among groups exception to LA+5. Thus, OCTT can be highly variable due to physiological differences. Such that a genetically longer esophagus, bigger stomach, or ability to secrete stomach acid and other reasons can potentially alter OCTT. Additionally, there are various time frames reported for breath hydrogen tests ranging from 7 hours to more than 28 hours (Ehrenpreis et al., 2002; van Munster et al., 1994).

In our study, we captured the rise from the 4th hour among all groups other than LA+5. Thus, we examined the AUC from 4th hour to distinguish the rise portion of the hydrogen

production. Even though we did not examine the significant difference within 4th hour AUC hydrogen, we noticed that the hydrogen production from MA+25 was much closer to the highest hydrogen production, LA+5. We could not conduct the statistical analysis due to not meeting normality test for 4th hour AUC hydrogen. Thus, if we extended the trials, we might have observed different results.

The PH of the colon is considered one physiological factor that can either be helpful or threatening for the growth of gut microbiota. Also, there are different routes that the hydrogen can take to be utilized, through hydrogenotrophs: methanogens, sulfate-reducing bacteria and acetogens (Nakamura et al., 2010). These process would consume produced hydrogen, and how much would be used remains a question. However, methanogenesis and sulfate reduction occur at neutral PH whereas acetogenesis perform best in an acidic PH (Gibson et al., 1990). If less active subject's colon PH were more acidic, there might have been more production of acetogens, however, if the PH is close to neutral (5.5-6.5), there may be more production of methane and hydrogen sulfide. Thus, it is difficult to examine the hydrogen concentration based on the PH of the colon. However, this might be highly interrelated with hydrogen production.

A recent study found that a greater body mass index (BMI) could potentially induced greater production of hydrogen. This clinical study discovered that within middle aged individuals, the ability to produce methane and hydrogen was associated with higher BMI. This study compared to other groups those are normal (< 20 ppm hydrogen and < 3 ppm methane producing individuals), hydrogen positive (≥ 20 ppm hydrogen, < 3 ppm methane) and methane positive (≥ 20 ppm methane, < 3 ppm hydrogen) groups (Mathur et al., 2013). The significance was found within both hydrogen and methane positive group. This is another piece of evidence that could reiterate the importance with the connection in between gut microbiota and body weight. Within

our study, the BMI one of the analysis on the descriptive variables did not show the significant difference among the 4 group ($p < 0.05$). However, LA+5 showed the highest hydrogen production. The BMI could also have factored in as one of the possible influences. The BMI of LA was considered overweight, thus, this may contribute the reason of why LA+5 was detected with second high breath hydrogen among groups. There were four who had BMIs > 25 in LA, whereas three had BMIs > 25 in MA. Also within BMI > 25 group, two had BMIs > 30 in LA and none in MA.

Limitation of breath hydrogen test

Previous clinical studies have examined the effect of resistant starch through breath hydrogen tests with controversial results. Some of the studies showed that there was no significant difference among breath hydrogen concentrations within healthy participants (Jenkins et al., 1998). Whereas elevated hydrogen production was detected in groups receiving high amounts of resistant starch (van Munster et al., 1994). We could not detect the difference of hydrogen production among groups. The possible reasoning for this would have been due to confounders such as not as diverse of physical activity, genetic, and dietary habit those might have been different among the groups. There were large inter-individual differences in our study, finding a method for decreasing this inter-individual differences could be helpful for the future study. Also, hydrogen can be produced within the small intestine and that would cause SIBO. This aspect was not properly captured, however, we did not have any subjects who were above 12ppm within first 2 hours of breath hydrogen response to be diagnosed with SIBO.

The mechanism behind breath hydrogen test is taken account of excreted air from lung and capture the transferred hydrogen via diffusion that was possibly produced from fermentation. In addition, in order to apply this methodology, two assumptions need to be satisfied: 1) the

production and consumption of H_2 should be at a constant with different doses and types of carbohydrate and 2) absorbed and excreted H_2 should be at a constant to the H_2 production. However, previous literature has found some errors involved in this method. There is a presence of a high variability among individuals' hydrogen production at baseline which was found within a study conducted with lactulose with different dosage through pulmonary H_2 excretion method (Bond & Levitt, 1972). Likewise, the differences among individuals can be weighted as significant factor in effecting the outcome. Possibly this could have outweighed the errors that were elicited from measurement error. Breath hydrogen test involves multiple assumptions to measure the hydrogen production, however, adapting this procedure may be the most practical in current science. In note, we examined methane, however, there was only few people who actually produced methane in either one feeding sessions. Thus, we decided to exclude them from examination. Overall, examining the pathway of hydrogen from the production to excretion *in vivo* may help to account for possible confounders and also for future advancement of the breath hydrogen test.

Experimental considerations

A major strength of this study is that it used a novel approach for examining the effect of chronic physical activity status and resistant starch type 4 intake on breath hydrogen production. To our knowledge, there has not been a study conducted to examine chronic physical activity and the intake of resistant starch type 4 in healthy young individuals. However, there are several important considerations to be taken into account when interpreting the results. To begin with, the measurement of breath hydrogen test, lacks in its accuracy of capturing the whole hydrogen produced from lung. Additionally, the hydrogen test has its drawback due to the test procedure that the subjects might have led to leak the exhaled hydrogen. However, in our study, the alveolar

sample was collected accordance to the instructions and guided verbally by the trained researcher. The syringe containing alveolar air was tightly closed after the subjects exhaled their breath into the tube and the syringe. Then, the trained researcher measured their breath within an hour to decrease the possibility of exhaled breath leaking from the syringe. Also, the breath hydrogen analyzer was always calibrated in timely manner prior to the trials to reduce any error caused from the machine.

Another consideration would be objectively measured physical activity status and IPAQ data collection. Even though the data were objectively measured with Actical, there is a possibility of a measurement error. In addition, completing the IPAQ required the subjects to recall their previous physical activity and also classifying moderate to vigorous physical activity can vary among the subjects. Specifically, subjects might not be able to quantify the time involved with constantly sitting and light walking within the office or other sedentary environments. Subjects often asked how they would categorize their daily walking minutes and overall activity at work or other physical labor on daily basis. This may be the reason why we had subjects misclassified at the beginning. However, we incorporated an objective measure for physical activity which is a strength of the current study.

Future directions

The results of the current study introduced further questions regarding the relationship with breath hydrogen production with physical activity and resistant starch intake. First, we observed a sustained glucose effect among MA. An increased plasma glucose level for the later minutes was expected due to the satiety effect of RS. However, there should be research that examines why this only occurred among MA and not LA. For future analyses, it would be valuable to divide LA based on steps whether they meet the recommendation of 10,000 per day or

BMI, whether they are above 25 or not. Also, considering possible confounders not previously discussed might be helpful for future research. Such confounders include age, insulin sensitivity, and OCTT. A larger sample size would be highly encouraged to account for such high variability observed in breath hydrogen.

Of note, if the current study was extended from 6 to 8 hours duration per trial, we might have different conclusions. It has been suggested that hydrogen production occurs at least 3-4 hours prior to consumption of the solid food, and some of the studies conducted more than 12 hours to examine the hydrogen production (Jenkins et al., 1998). Thus, if we had extended the duration of the trials, MA+25 might have shown significantly greater hydrogen production as compared to the other groups. However, we did not detect any effect from PA or RS intake. The combination of chronic physical activity and resistant starch type 4 has not been explored in the past, thus, this field of study is waiting for the next step.

Conclusions

The present study did not observe an affect of chronic physical activity and acute resistant starch intake on hydrogen production. However, within postprandial glucose response, we did detect a difference within the more active group. Specifically, among MA, a sustained glucose effect was observed with RS. Thus, increased RS may help to stabilize blood glucose a little better over time in those who are more active. Hydrogen production, as an indirect measure of gut fermentation, is complex and future work in this area should consider including a standardized meal a day prior to the measurement of breath hydrogen, control or standardize intake of fermentable dietary fiber, measure breath hydrogen for longer than six hours, and use reliable and valid PA measures. Last but not least is considering collecting more samples and recruiting more subjects within diverse groups to increase the diversity of PA status within the groups. Thus,

based on the results of the present study, it is recommended to consider these variables in future studies examining the effects of PA and RS on metabolic and gut health outcomes.

Reflection

During this master's program in nutrition at Kansas State University, the courses that I took ignited my interest within nutrition even deeper and further. Being here in America, I was awed by the novel information that I encountered on daily basis. Especially the macronutrient class with Dr. Melgarejo that I took on the first semester where I learned how to conduct and to theorize the research. The topic with gut microbiota was considered novel and highly interesting, which is also related to my interest within type 2 diabetes and carbohydrate intake including fiber. Also, the statistics class helped me to understand how to model the study and be able to quantify the results in numeric form for the public to understand. It was another challenge for me to comprehend a theory that I never was exposed. Even though I conducted a research as an assistant during my undergraduate year, I did not know exactly why and how the statistics worked. Overall, graduate classes were overwhelmed but just in a good way that I could be challenged and overcome to gain more knowledge.

Moreover, the most life changing experience that I had from Kansas State University would be heavily accounted upon PAN-CRC (Physical Activity and Nutrition Clinical Research Consortium). I would truly say that the meetings that I had in PAN-CRC every week, it was always something new -- whether it was scientific or more of the general information that was beneficial for young scientists. What I found the most different about my country and for the education system is that, here at PAN-CRC, we were all treated as a scientist and were trained to be the one who could contribute the science and the finding into society. It is often very rare to find the environment where the science could be treated in a generous and critical manner for its

applicability to society. From here, I learned how to gratefully comprehend the science and simultaneously criticize the piece of art. There was the phase of each member representing their science during the lab and I was trying to convey the message from their presentations even though the interest and the method could not be applied to my study or the interest of my research. Acknowledging the values of different fields of science truly touched my heart to comprehend the broader picture and the value of each projects. Regardless of their finding, the procedure and the effort that each member at the PAN-CRC put was admirable.

Most importantly, the topic of gut microbiota relates to RS and PA opened my view further into science. The procedure and creating a protocol were valuable experiences. Based on my previous experiences, this work at K-State truly aided me to be one step closer to being an independent researcher. Without knowing it, my academic experience (almost 6 years) helped developed what I truly am interested in. From recruiting participants, dealing with the withdrawal of participants, communicating with participants before their trials, and staying with them from morning through the afternoon has taught me how much work is required to complete studies like this. By conducting this study, I, also, learned how to connect with participants. Through daily communication, I learned the eating habits of 20 participants, as well as their lifestyles and interests about being healthy, which I believe it was an immeasurable experience. However, with all due respect to those benefits, I also, learned that dealing with human subjects is not as convenient as what I originally assumed. For example, participants would come in for the study and then learn that they were not qualified to participate even though I acknowledged they were based on information they provided in emails. Or, they might email me early in the morning on the actual trial day to inform me that they cannot make it.

However, without this study, I would not have understood fully the importance of the data collection process and how to deal with certain situation where I would take responsibility and have a decision-making role. Moreover, with data analysis, it was a great help that the school supported students by providing access to statistic programs at PAN-CRC. Additionally, without the help from the nutrition epidemiology class (taught by Dr. Ric Rosenkranz), advice from a former statistics professor and members from PAN-CRC (especially Dr. Sara Rosenkranz), and input from my major professor, Dr. Haub, it would have been much more difficult. Overall, it was the work that I believe I would not have accomplished without the help from K-State and PANCRC. There are remaining areas where I hope to improve. However, this process strengthened my interest towards research focused on nutrition.

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Appendix A - Individual graph and figures

Postprandial glucose iAUC and breath hydrogen AUC

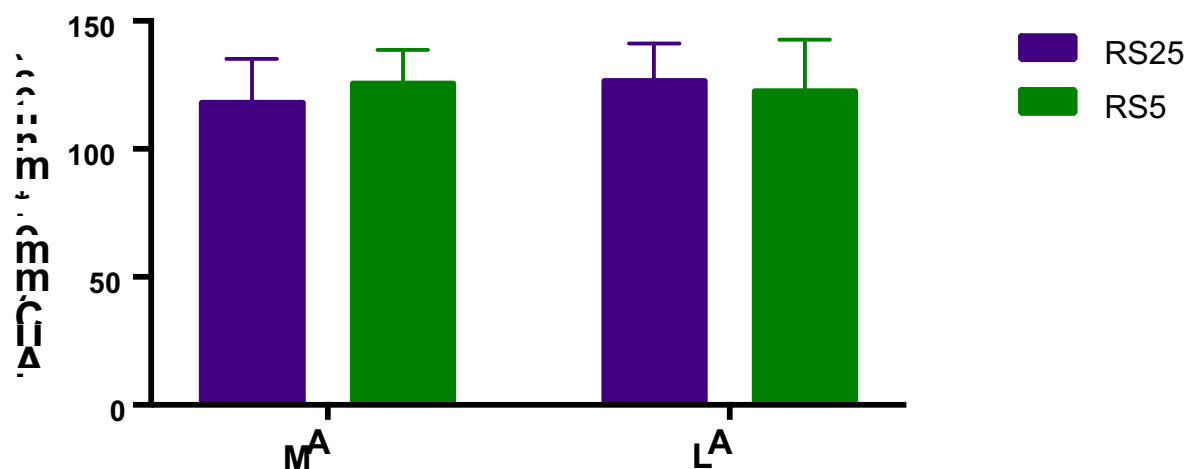


Figure A.1 incremental area under the curve (iAUC) glucose response among MA and LA

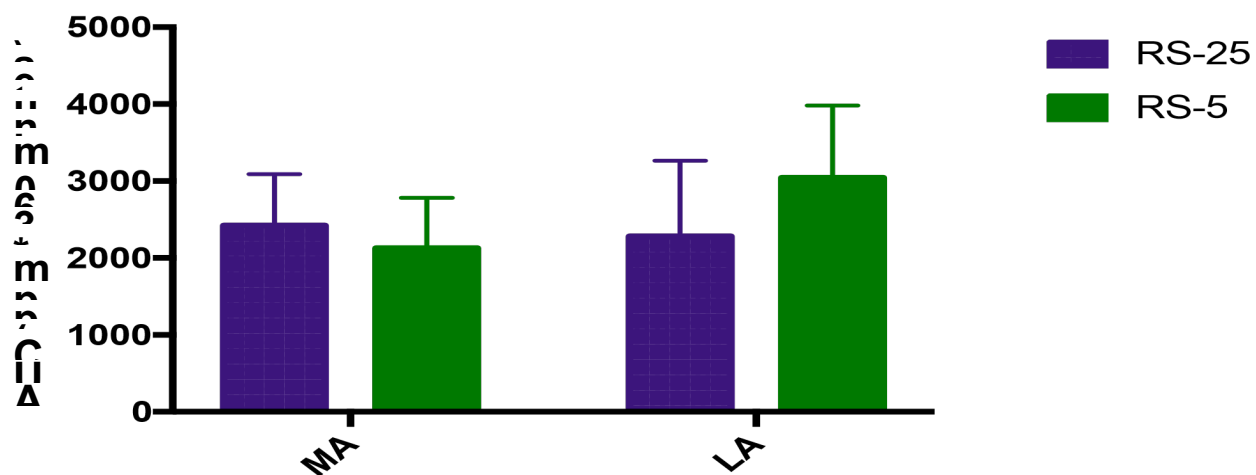
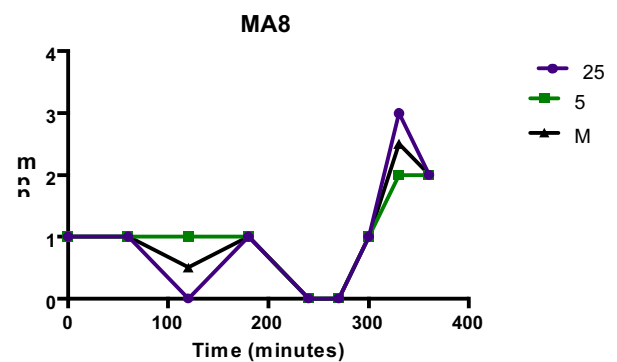
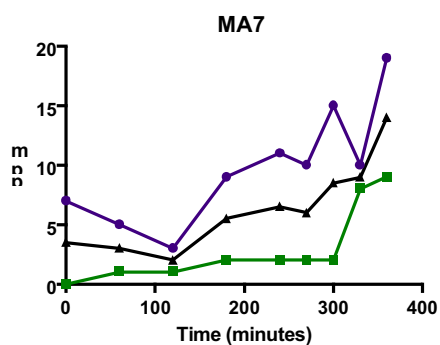
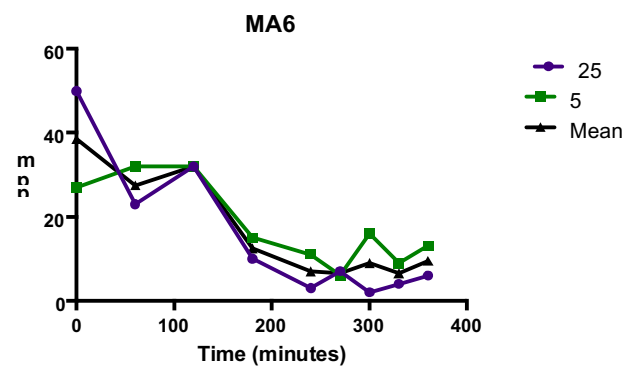
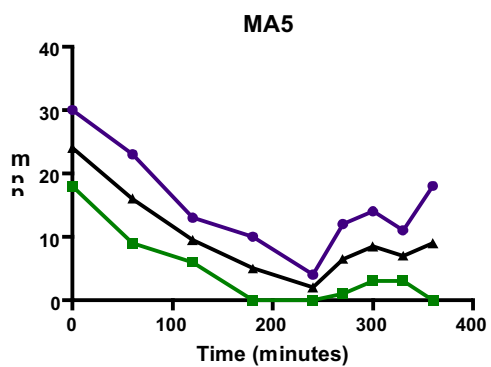
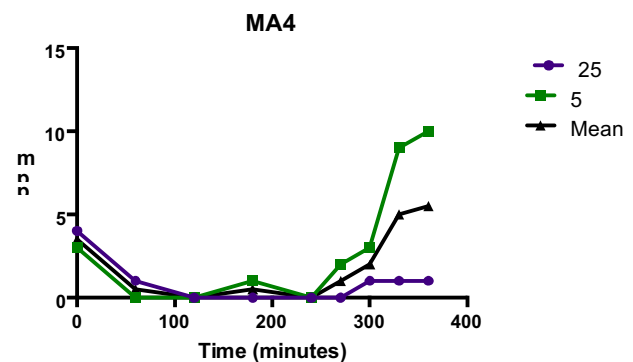
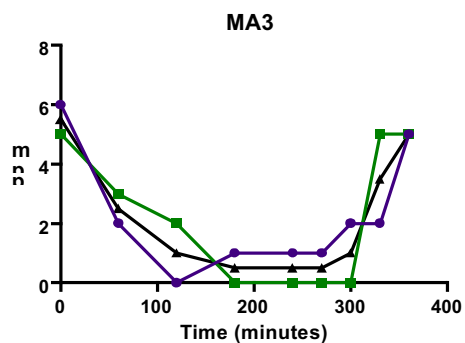
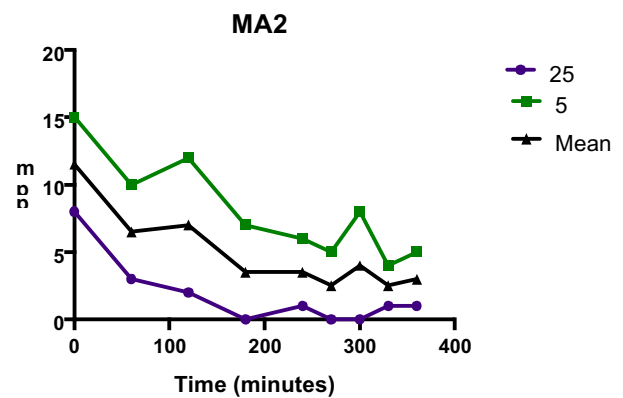
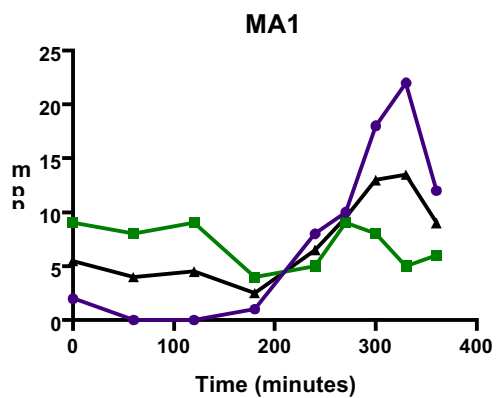


Figure A.2 area under the curve (AUC) breath hydrogen response among MA and LA

Postprandial glucose and breath hydrogen response in individual graph



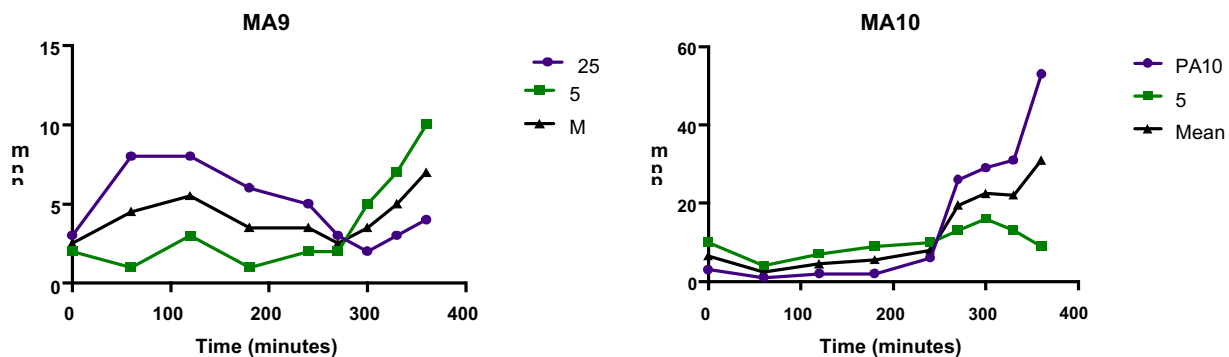
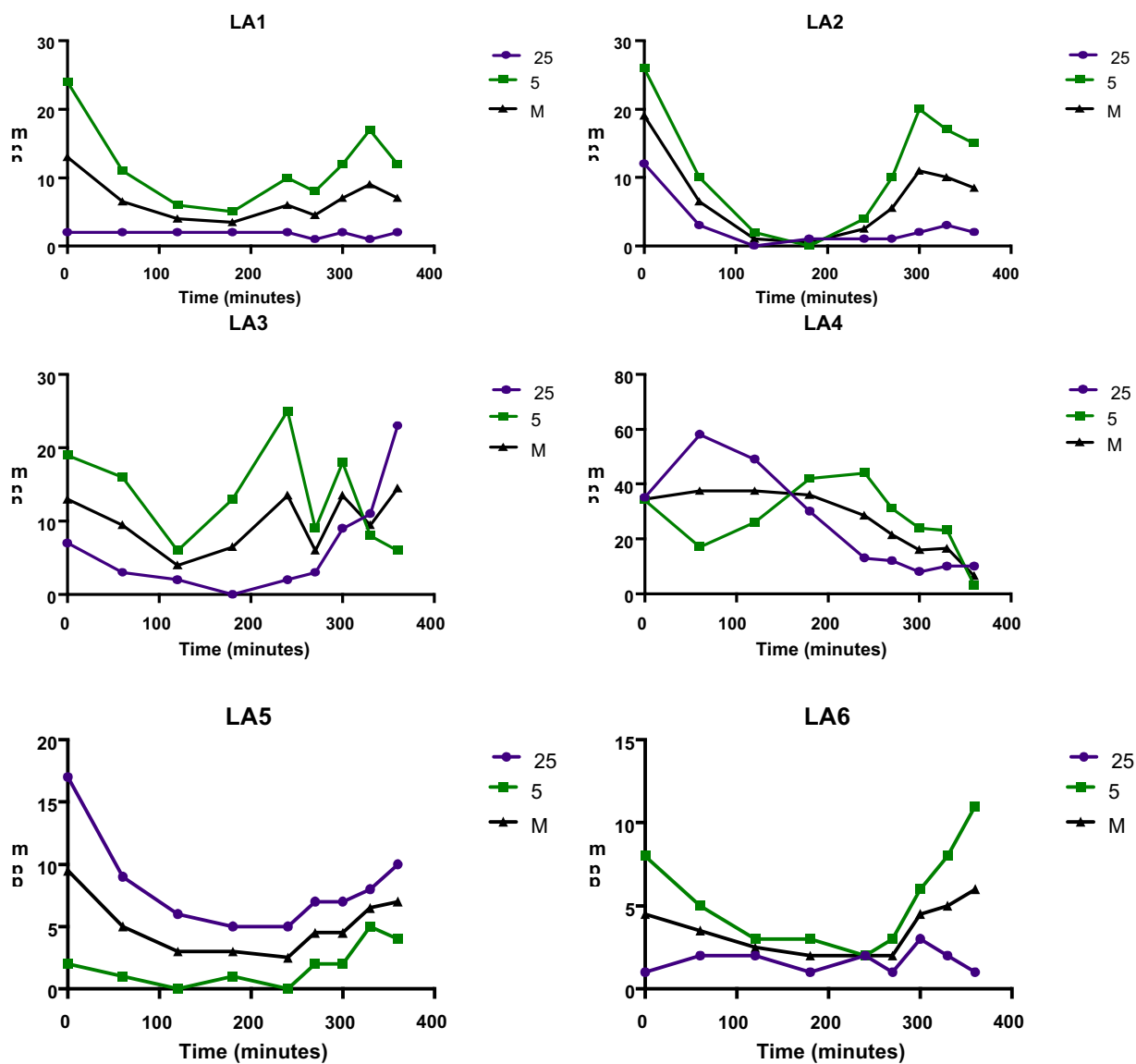


Figure A. 3 Individual breath hydrogen response among MA



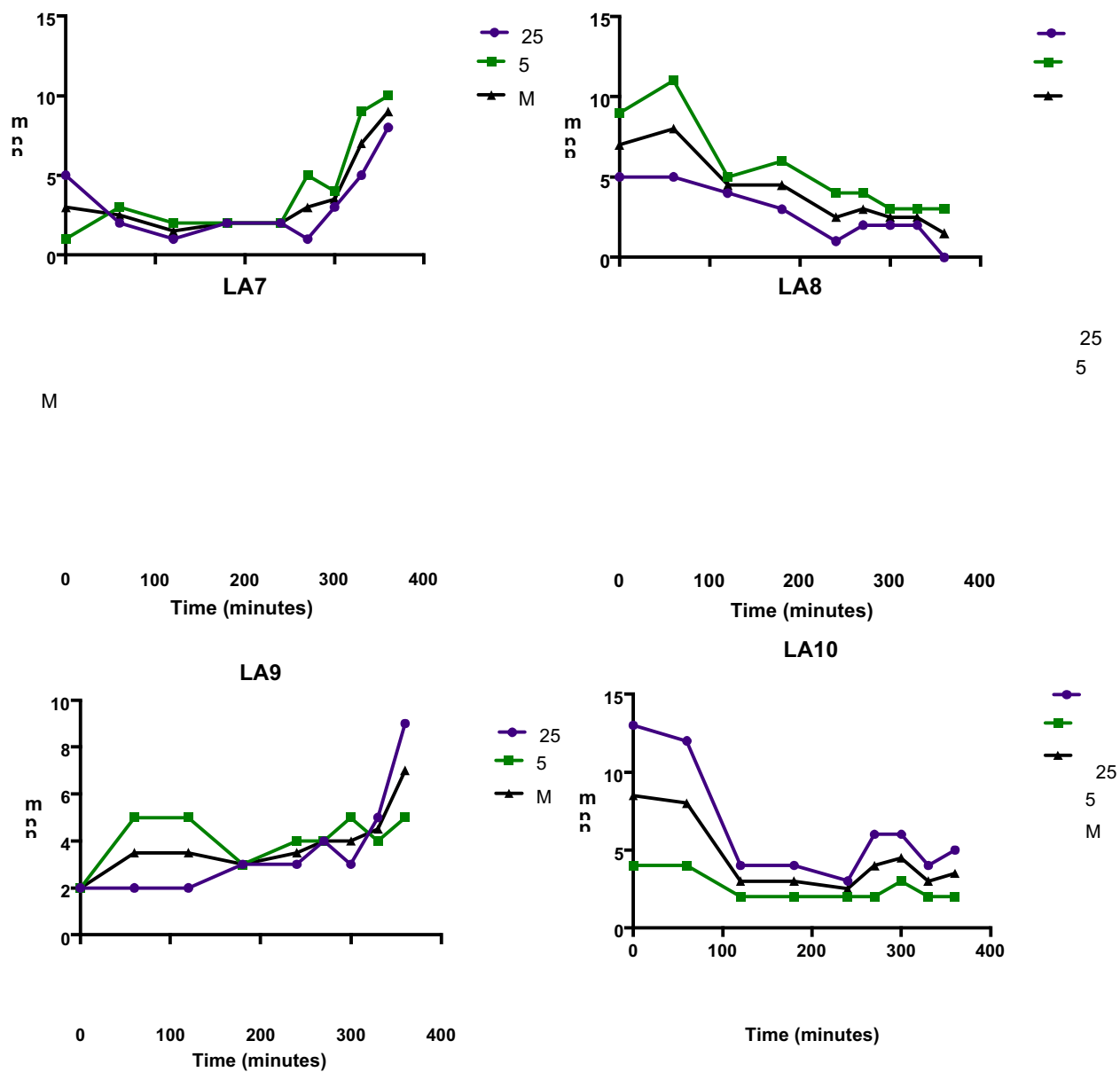


Figure A.4 Individual breath hydrogen response among LA