EFFECTS OF DIET INDUCED SHORT CHAIN FATTY ACIDS ON BLOOD METABOLITES AND KEY REGULATORS OF LIPID METABOLISM IN GILTS

by

JAMES M. LATTIMER

B.S., Kansas State University, 2002
M.S., Oklahoma State University, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Human Nutrition
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2012

Abstract

Background: Dietary fiber has been shown to help improve several metabolic disorders including obesity and type II diabetes. However, the mechanism by which this occurs is poorly understood.

Purpose: This study was designed to compare the effects of energy restriction and dietary fiber and subsequent production of short chain fatty acids on body composition, biomarkers of health, and hepatic and myocellular expression of key regulators of lipid metabolism

Methods: Crossbred gilts (n=17) were randomly assigned to either a control (CON), high fiber (HF) or energy restricted (ER) diet for 42 days. Gilts on the CON and HF diets were fed ad libitum. The ER Gilts were pair fed HF gilts and matched for body weight gain. Blood samples were collected and glucose, insulin, triglycerides, non-esterified fatty acids and short chain fatty acids (SCFA) concentrations were measured. Liver and muscle tissue were biopsied and expression of peroxisome proliferator-activated receptor gama (PGC-1α) and carnitine palmitoyltransferase 1 (CPT1) were determined via RT-PCR.

Results: HF gilts had significantly higher plasma TG and lower NEFA concentrations when compared to the CON and ER. The HF diet elicited a significant increase in all plasma SCFA concentrations. No differences in fold change of myocyte CPT1 and PGC-1α mRNA expression were found while they tended to be lower in hepatic samples of the HF gilts. HF gilts also had a lower ($P < 0.05$) back fat thickness when compared to the ER even though energy intakes were similar. Minimal changes were observed in fasting glucose and insulin as a result of diet.

Conclusions: Gilts consuming a diet high in dietary fiber (DF) significantly altered their plasma lipid profiles independently to that of energy restriction and body weight and appears to be a result of plasma SCFA concentration. DF and/or SCFA appear to have minimal affects on CPT1 and PGC-1α in the liver and muscle of gilts.

EFFECTS OF DIET INDUCED SHORT CHAIN FATTY ACIDS ON BLOOD METABOLITES AND KEY REGULATORS OF LIPID METABOLISM IN GILTS

by

JAMES M. LATTIMER

B.S., Kansas State University, 2002
M.S., Oklahoma State University, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Human Nutrition
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2012

Approved by:

Major Professor
Mark D. Haub

Copyright

JAMES M. LATTIMER

2012

Abstract

Background: Dietary fiber has been shown to help improve several metabolic disorders including obesity and type II diabetes. However, the mechanism by which this occurs is poorly understood.

Purpose: This study was designed to compare the effects of energy restriction and dietary fiber on body composition, biomarkers of health, and hepatic and myocellular expression of key regulators of lipid metabolism

Methods: Crossbred gilts (n=17) were randomly assigned to either a control (CON), high fiber (HF) or energy restricted (ER) diet for 42 days. Gilts on the CON and HF diets were fed ad libitum. The ER Gilts were pair fed HF gilts and matched for body weight gain. Blood samples were collected and glucose, insulin, triglycerides, non-esterified fatty acids and short chain fatty acids (SCFA) concentrations were measured. Liver and muscle tissue were biopsied and expression of peroxisome proliferator-activated receptor gama (PGC-1α) and carnitine palmitoyltransferase 1 (CPT1) were determined via RT-PCR.

Results: HF gilts had significantly higher plasma TG and lower NEFA concentrations when compared to the CON and ER. The HF diet elicited a significant increase in all plasma SCFA concentrations. No differences in fold change of myocyte CPT1 and PGC-1α mRNA expression were found while they tended to be lower in hepatic samples of the HF gilts. HF gilts also had a lower ($P < 0.05$) back fat thickness when compared to the ER even though energy intakes were similar. Minimal changes were observed in fasting glucose and insulin as a result of diet.

Conclusions: Gilts consuming a diet high in dietary fiber (DF) significantly altered their plasma lipid profiles independently to that of energy restriction and body weight and appears to be a result of plasma SCFA concentration. DF and/or SCFA appear to have minimal affects on CPT1 and PGC-1α in the liver and muscle of gilts.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would first like to thank Dr. Mark Haub for his guidance and support throughout my doctoral work. He allowed diversity in my research/coursework and supported my inter-departmental relationships. As a result of his mentoring, my fellowship was truly unique and one not commonly found among graduate students.

My committee, including Dr. Tona Melgarejo, Dr. Y.C Shi and Dr. Jim Nelssen, also deserves full acknowledgements for their support and leadership. Without them the success of my research as well as my professional future would surely be hindered.

I would like to extend much appreciation to all others who assisted me throughout my fellowship. My fellow graduate students, including Casey Weber, Tara Lopez and Matt Asmus, were a huge asset in my research and laboratory responsibilities. Dr. Joel DeRouchey, Dr. David Anderson, Dr. Barry Bradford, Dr. Laman Mamedova and Cheryl Armendariz were also key members of my research. Their contributions are greatly appreciated.

Last but not least, I would like to thank my wife and parents for their love, support and sacrifices. Without them I could not have succeeded.

Dedication

I dedicate this work to my wife Nichole L. Lattimer

Without her support and sacrifice my fellowship would not have been possible

Preface

Chapter 1, entitled “Effects of Dietary Fiber and its Components on Metabolic Health” was published in Nutrients, 2(12), 1266-1289. This journal required a numbering format when citing references. Chapter 2, however, will be submitted to The Journal of Nutrition which requires ASA style formatting of references. Therefore, the two chapters will have their own individual list of references in the format dictated to by their respective journal.

Chapter 1 - Effects of Dietary Fiber and its Components on Metabolic Health

Lattimer, J.M. and M.D. Haub. 2010. Effects of Dietary Fiber and its Components on Metabolic Health. Nutrients 2(12):1266-1289.

Introduction

Dietary fiber and whole grains contain a unique blend of bioactive components including resistant starches, vitamins, minerals, phytochemicals and antioxidants. As a result, research regarding their potential health benefits has received considerable attention in the last several decades. Epidemiological and clinical studies demonstrate that consumption of dietary fiber and whole grain intake is inversely related to obesity [1], type two diabetes [2], cancer [3]and cardiovascular disease (CVD) [4].

The Food and Drug Administration (FDA) has approved two health claims for dietary fiber. The first claim states that, along with a decreased consumption of fats (<30% of calories), an increased consumption of dietary fiber from fruits, vegetables and whole grains may reduce some types of cancer [5]. "Increased consumption" is defined as six or more one ounce equivalents with three ounces derived from whole grains. A one ounce equivalent would be consistent with one slice of bread, ½ cup oatmeal or rice, or five to seven crackers.

Recent studies support this inverse relationship between dietary fiber and the development of several types of cancers including colorectal, small intestine, oral, larynx and breast [3,6,7]. Although most studies agree with these findings, the mechanisms responsible are still unclear. Several modes of actions however have been proposed. First, dietary fiber resists digestion in the small intestine, thereby allowing it to enter the large intestine where it is fermented to produce short chain fatty acids, which have anti-carcinogenic properties [8].

Second, since DF increases fecal bulking and viscosity, there is less contact time between potential carcinogens and mucosal cells. Third, DF increases the binding between bile acids and carcinogens. Fourth, increased intake of dietary fiber yield increased levels of antioxidants. Fifth, DF may increase the amount of estrogen excreted in the feces due to an inhibition of estrogen absorption in the intestines [9]

The second FDA claim supporting health benefits of DF states that diets low in saturated fat (<10% of calories) and cholesterol and high in fruits, vegetables and whole grain, have a decreased risk of leading to coronary heart disease (CHD) [10]. For most, an increased consumption of dietary fiber is considered to be approximately 25 to 35 g/d, of which 6 g are soluble fiber.

Obviously, many studies support the inverse relationship of dietary fiber and the risk for CHD. However, more recent studies found interesting data illustrating that for every 10 g of additional fiber added to a diet the mortality risk of CHD decreased by 17-35% [11,12]. Risk factors for CHD include hypercholesterolemia, hypertension, obesity and type two diabetes. It is speculated that the control and treatment of these risk factors underlie the mechanisms behind DF and CHD prevention. First, soluble fibers have been shown to increase the rate of bile excretion therefore reducing serum total and LDL cholesterol [13]. Second, short chain fatty acid production, specifically propionate, has been shown to inhibit cholesterol synthesis [14]. Third, dietary fiber demonstrates the ability to regulate energy intake thus enhancing weight loss or maintenance of a healthier body weight. Fourth, either through glycemic control or reduced energy intake, dietary fiber has been shown to lower the risk for type two diabetes. Fifth, DF has been shown to decrease pro-inflammatory cytokines such as interleukin–18 which may have an

effect on plaque stability [15]. Sixth, increasing DF intake has been show to decrease circulating levels of C-Reactive protein (CRP), a marker of inflammation and a predictor for CHD [16].

Although only two claims have been adopted and supported by the FDA, multiple other "potential claims" have been researched and well documented. Those conditions of particular importance, due to their increasing prevalence among the general population, include obesity and type two diabetes [17,18]. The digestive physiology of dietary fiber has significant implications in the risk for and treatment of these metabolic disorders. Dietary fibers have been shown to result in decreased blood glucose excursions and attenuated insulin responses. This may be due to either a delayed [19] or decreased [1] intestinal absorption. Therefore, the purpose of the paper is to review current research regarding dietary fiber and whole grains in relation to these proposed claims. Focus will be placed on nutrient absorption, postprandial glycemia, insulin sensitivity, caloric density and satiety. Furthermore, select constituents of DF will be discussed in detail to better their role on metabolic health.

Defining Dietary Fiber

In the simplest form, carbohydrates can be separated into two basic groups based upon their digestibility in the GI tract. The first group (i.e starch, simple sugars, and fructans) is easily hydrolyzed by enzymatic reactions and absorbed in the small intestine. These compounds can be referred to as non-structural carbohydrates, non-fibrous polysaccharides (NFC) or simple carbohydrates. The second group (i.e. cellulose, hemicellulose, lignin, pectin and beta-glucans) are resistant to digestion in the small intestine and require bacterial fermentation located in the large intestine. These compounds can be referred to as complex carbohydrates, non-starch polysaccharide (NSP) or structural carbohydrates and are reflective in NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) analysis. NDF consists of cellulose, hemicelluloses and

lignin while ADF consists of cellulose and lignin. However, NDF and ADF analysis are used primarily for animal nutrition and the analysis of roughages.

Although, NDF and ADF are typically not used in human nutrition, the separation of structural (NSP) and non-structural carbohydrates provide the basis for beginning to define and understand dietary fiber. This task has been a divergent process and has depended on both nutrition and analytical concepts. The most common and accepted definition is based on nutritional physiology. However, chemists and regulatory boards have leaned more toward analytical procedures to define dietary fiber on fact. The physiological definition is easier for the general public to understand and place its definition in a practical use.

American Association of Cereal Chemists

A recent description, as suggested by the American Association of Cereal Chemists [20], terms dietary fiber as carbohydrate polymers with more than a 3 degree polymerization which are neither digested nor absorbed in the small intestine. The greater than 3 degree polymer rule was designed to exclude mono and disaccharides. The known constituents of dietary fiber are listed in Table 1.

This definition describes in more detail the component of dietary fiber as well as its genetic makeup. Furthermore, the changes set forth in its description require few changes for its analytical evaluation [21].

World Health Organization and Food and Agriculture Organization

The World Health Organization (WHO) and Food and Agriculture Organization (FAO) agree with the AACC definition but with a slight variation. They state that dietary fiber is a polysaccharide with ten or more monomeric units which is not hydrolyzed by endogenous hormones in the small intestine [22].

Soluble vs. Insoluble

NSP can be further subdivided into the two general groups of soluble and insoluble. This grouping is based on chemical, physical, and functional properties [24]. Soluble fiber dissolves in water forming viscous gels. They bypass the digestion of the small intestine and are easily fermented by the microflora of the large intestine. They consist of pectins, gums, inulin-type fructans and some hemicelluloses. In the human GI tract, insoluble fibers are not water soluble. They do not form gels due to their water insolubility and fermentation is severally limited. Some examples of insoluble fiber are of lignin, cellulose and some hemicelluloses. Most fiber containing foods include approximately one-thirds soluble and two-third insoluble fiber [25].

Proposed Health Benefits of Dietary Fiber

Dietary fiber and whole grains are an abundant source of nutrients including vitamins, minerals, and a slowly digestible energy. In addition, they contain phytochemicals such as phenolics, carotenoids, lignans, beta-glucan and inulin. These chemicals, secreted by plants, are not currently classified as essential nutrients but may be important factors in human health [26]. The synergistic effect of phytochemicals, increased nutrient content and digestive properties are believed to be the mechanism behind dietary fibers beneficial effects on the treatment and prevention of obesity and diabetes [1,27], reduced CVD [28] and decreased incidence of certain types of cancer [29,30]. In the following subsections, potential health benefits of dietary fiber will be reviewed along with their possible mechanisms and modes of actions.

Obesity

Approximately 66% of US adults are overweight or obese [17] resulting in an increased risk of health problems including, but not limited to, diabetes, CVD, and certain types of cancer [31]. Although there are multiple factors that could contribute to obesity, the primary cause is due to an increase in the energy absorption:energy expenditure ratio. Therefore, limiting energy absorption is critical when treating obesity. Scientists have taken this a step further and studied the effect of other dietary aspects that may serve in weight regulation, including dietary fiber. Increasing dietary fiber consumption may decrease energy absorption by way of diluting a diet's energy availability while maintaining other important nutrients.

Substantial research has been conducted to evaluate the effect of dietary fiber and body weight, most all of which show an inverse relationship between dietary fiber intake and change in body weight. Tucker and Thomas [1] supported this statement in a study consisting of 252 middle aged women. They observed that over a 20 month period participants lost an average of 4.4 lbs due to an 8 g increase in dietary fiber per 1000 kcal. This weight loss was primarily due to decreased body fat. It should be recognized that the correlation between dietary fiber and weight change was independent of many other potential factors including age, baseline fiber and fat intakes, activity level, and baseline energy intake.

Koh-Banerjee *et al.* [32] concur with the above findings and also suggest a dose-response relationship. They reported that for every 40 g/d increase in whole grain intake, weight gain decreased by 1.1 lbs. Moreover, bran seemed to play an important role in the reduction of weight gain by 0.8 lbs per 20 g/d intake.

Dietary fiber's ability to decrease body weight or attenuate weight gain could be contributed to several factors. First, soluble fiber, when fermented in the large intestine, produces

glucagon-like peptide (GLP-1) and peptide YY (PYY) [33]. These two gut hormones play a role inducing satiety. Second, dietary fiber may significantly decrease energy intake [1]. Women who consumed increased levels of fiber tended to also have a decreased consumption of dietary fat. Third, dietary fiber may decrease a diets metabolizable energy (ME), which is gross energy minus the energy lost in the feces, urine and combustible gases. Baer *et al.* [34] observed that an increase consumption of dietary fiber resulted in a decrease in the ME of the diet. This may be attributed to the fact that fat digestibility decreased as dietary fiber increased. Also, as dietary fiber intake increases the intake of simple carbohydrates tends to decrease. Although, dietary fiber still contributes to the total caloric content of a diet, it is much more resistant to digestion by the small intestine and even somewhat resistant in the large intestine.

It should also be noted that the inverse relationship between dietary fiber and ME was independent of dietary fat. Therefore, ME decreased as dietary fiber increased in both a high and low fat diets. However, when dietary fiber was split into soluble and insoluble fiber, the results were much more inconclusive. Soluble fiber decreased ME when added to a low fat diet but increased ME when added to a high fat diet [34]. It is not really known how dietary fat changes the effects of soluble fiber. Isken *et al.* [35] showed supportive data in mice consuming a high fat diet. Mice showed an increased weight gain when soluble fiber was added to a high fat diet. There are several mechanisms that may explain how soluble fiber could increase ME or weight gain. First, bacterial populations in the large intestine increase due to an increase in soluble fiber consumption [36]. This could result in an increased fermentation and utilization of short chain fatty acids thereby increasing energy absorption. Second, soluble fiber enlarges in the GI and forms a viscous material which delays intestinal transit time [37]. Subsequently, this increase time in the GI tract may allow for more complete digestion and absorption. Conversely, some

believe this increase viscosity has an opposite effect and retards absorption [27]. More research is needed in this area.

Insoluble fiber seems to have the opposite effect to that of soluble. When insoluble fiber intake was increased in mice consuming a high fat diet, body weight decreased [35]. Research in sows demonstrated that insoluble fiber decreased energy digestibility while it increased with soluble fiber intake [38]. The mode of action behind these findings may be due to the fact that insoluble fiber causes an increased rate of passage through the GI tract [39]. This would be expected to result in diminished digestion and absorption of nutrients.

According to the data presented above, both soluble and insoluble fiber may lead to weight loss. However, there seems to be a relationship between the type of diet (high or low fat) and the type of fiber consumed. Insoluble fiber may play a more important role for weight loss during consumption of a high fat diet. Since resistant starch is a constituent of dietary fiber and undergoes the same digestion as insoluble fiber, comparing resistant starch and insoluble fiber may give us a better understanding of how dietary can be used to treat and prevent obesity. Adding resistant starch to a diet dilutes its ME, but not to the degree of insoluble fiber [40].

Numerous studies [32,41] have found the same inverse relationship between dietary fiber and weight gain. However, the data are more inconsistent when comparing soluble and insoluble. Thus, although increasing dietary fiber in general has favorable effect on body weight, more research is warranted to determine the optimal dietary fibers for the purposes of weight management.

Diabetes

Type 2 diabetes has increased exponentially over the past several years. Since 1990, self reported diabetes increased 61% [18]. Although other risk factors such as obesity, lack of

physical activity and smoking are precursors for the disease, dietary factors also seem to play a significant role. Type two diabetes results from decreased insulin sensitivity and hyperglycemia. For that reason, a primary dietary factor of particular concern is carbohydrate intake.

Meyer *et al.* [42] observed that total carbohydrates had no effect on the risk of diabetes. However, the type of carbohydrate (non structural carbohydrates and dietary fiber) was significantly related. Therefore, it is important to understand a foods glycemic index or load. Glycemic index ranks total carbohydrate intake according to their immediate postprandial glucose response in comparison to a reference group such as glucose or white bread. Carbohydrates with a low glycemic index result in a smaller glucose/insulin response. Simple small chain carbohydrates would be considered to have a higher glycemic index since it produces higher blood glucose concentrations.

While Hu *et al.* [43] found that excess body fat was the single most important determinant of type two diabetes, poor nutrition was also a large influential factor. Moreover, women consuming a poor diet significantly increased their risk of developing type 2 diabetes. Poor diet was classified as a diet high in saturated fat, low dietary fiber and high non structural carbohydrates. This diet would be consistent with a high glycemic load being higher in easily digestible and rapidly absorbable carbohydrates. In a supportive, long term (8 yr) study of over 90,000 female nurses, Shuzle *et al.* [44] found a positive correlation between glycemic index and risk of type two diabetes. This association was significant even after adjusting for age, body mass index (BMI) and family history. Several means have been proposed to understand the physiology behind the relationship of glycemic index and diabetes. First, carbohydrates with a higher glycemic index produce higher blood glucose levels. This chronic hyperglycemia is suggested to lead to the dysfunction of beta cells in the pancreas thus decreasing insulin release.

Second, due to an over abundance of energy (i.e. high glycemic load) tissues such as skeletal muscle, liver and adipose become resistant to insulin.

Although a majority of studies show a positive correlation between high glycemic foods and type two diabetes, several studies disagree with these findings. Meyer *et al.* [42] found that glycemic index had no effect on the prevalence of diabetes in older aged women. However, there was a strong ($P = 0.005$) inverse relationship between dietary fiber intake and diabetes when adjusted for age and BMI. Women consuming an average of 26 g/d of dietary fiber had a 22% lower risk of developing diabetes when compared to women only consuming 13 g/d. Schulze *et al.* [44] agreed with these findings with men and women showing a decrease risk of diabetes ($P<.001$) with the consumption of an additional 12 g of dietary fiber per day. According to these findings, it may be more significant to focus on an increased consumption of dietary fiber to prevent diabetes than glycemic index/load.

It is important to note that the inverse relationship between dietary fiber and diabetes observed by Meyer *et al.* [42] and Schulze *et al.* [44]was independent of age and body weight. Hu *et al.* [43] supported these findings while correcting for age, fat intake, smoking, alcohol, family history, exercise, and body weight. Therefore, it seems that dietary fiber provides is associated with type two diabetes independent of other compounding factors.

According to recent research, the soluble *vs.* insoluble fraction of fiber may give some insight on the efficacy of dietary fiber on diabetes and its mechanisms. Early research regarding soluble fiber demonstrated delayed gastric emptying and decreased absorption of macronutrients resulting in lower postprandial blood glucose and insulin levels [45]. This is most likely due to the viscosity of soluble fibers inside the GI tract. Interestingly, different types of soluble fiber had varying effects on viscosity and nutrient absorption. Guar had the highest viscosity as well as

the greatest effect at decreasing postprandial blood glucose. Therefore, it would be assumed that an increased level of soluble fiber would be associated with a decrease risk of diabetes. However, several recent studies have demonstrated the opposite showing no correlation between soluble fiber and a reduced risk of diabetes [42,44,46].

Although some studies have been contradictory, showing no differentiation between soluble and insoluble fiber on diabetes [44], a majority of the research demonstrates a strong inverse relationship between insoluble fiber and the risk of type 2 diabetes. Meyer *et al.* [42], using healthy middle aged women, observed a strong (P=.0012) inverse relationship between insoluble fiber and the risk of type 2 diabetes while soluble fiber had no effect. Montonen *et al.* [46] also found the same results in healthy middle aged men and women consuming increased levels of whole rye bread. Interestingly, fiber from fruits and vegetables had no effect on the risk of developing type 2 diabetes. Earlier studies have agreed with these findings. A large epidemiological study of 42,000 men found that dietary fiber from fruits or vegetables had no effect on the risk of diabetes. However, dietary fiber from whole cereal grains showed a significant decrease in diabetes occurrence [47]. Daily intakes of fiber among all groups were similar.

Insoluble fiber only has a small effect on macronutrient absorption [45]. Therefore, another mode of action must be present and several hypotheses should be discussed. Some suggest that insoluble fiber increases the passage rate of foodstuff through the GI tract thus resulting in a decreased absorption of nutrients, namely simple carbohydrates. However, Weicket *et al.* [27] found that an increased intake of cereal fiber significantly improved whole body glucose disposal resulting in an 8% improvement of insulin sensitivity. This suggests that the mechanisms behind insoluble fiber are more peripheral and not limited to nutrient absorption.

First, an accelerated secretion of glucose-dependent insulintropic polypeptide (GIP) was observed directly after the ingestion of an insoluble fiber in healthy women [48]. GIP is an incretin hormone which stimulates postprandial insulin release. Second, insoluble fiber can result in a reduced appetite and food intake [49]. This may lead to a decreased caloric intake and BMI as described in the obesity section of this review. Third, short chain fatty acids, via fermentation, have been shown to reduce postpandrial glucose response [50,51]. Early research demonstrated that lipid infusions impaired glucose utilization [52] and oral acetate could decrease free fatty acids (FFA) in the blood [53]. According to Kelley and Mandarino [54], increases in FFA in the blood can inhibit glucose metabolism through the inhibition of GLUT 4 transporters. Therefore, short chain fatty acids, by way of decreasing serum free fatty acids, may reduce blood glucose levels through competition in insulin-sensitive tissues.

The inverse relationship between cereal grains and diabetes may also be attributed to an increased consumption of Magnesium. Increased intake of magnesium has been shown to decrease the incidence of type 2 diabetes [42,55]. Hypomagnesemia is common among diabetics and has been associated with a reduction of tyrosine kinase at the insulin receptor [56]. This may impair the action of insulin thus leading to insulin resistance.

According to recent research, increasing levels of dietary fiber may contribute a non-pharmacological way to improve carbohydrate metabolism. However, some inconsistency does exist and may be contributed to the classification of dietary fiber and whole grains. Subjects with diagnosed type 2 diabetes may also add to the irregularity among the data. In a study of men and women with established diabetes, Jenkins *et al.* [57] observed that wheat bran had no effect on glycemic control in subjects with type 2 diabetes. Much of the research showing improved glycemia with dietary fiber examined health subjects, rather than subjects with diagnosed type

two diabetes. Further research is needed to determine whether dietary fiber may be able to aid in the control of normal blood glucose levels in subjects with established type 2 diabetes.

Fiber Components of Interest

Dietary fiber can be separated into many different factions (Table 1.). Recent research has begun to isolate these components and determine if increasing their levels in a diet is beneficial to human health. The separation of these fractions may give us a better understanding of how and why dietary fiber may decrease the risk for certain diseases.

Arabinoxylan

Arabinoxylan (AX), a constituent of hemicelluloses, is comprised of a xylose backbone with arabinose side chains. AX is a major component of dietary fiber in whole grains having considerable inclusions in both the endosperm and bran. In wheat, AX account for around 64 – 69% of the NSP in the bran and around 88% in the endosperm [58]. During normal wheat flour processing, a majority of the AX is removed as a by-product. In the GI tract AX acts much like a soluble fiber being rapidly fermented by the micrflora of the colon.

Lu *et al.* [59], observed an inverse relationship between the intake level of an AX rich bread and postprandial glucose response in healthy adult subjects. When compared to the control, postprandial glucose levels were significantly lower with only 6 g of AX rich fiber supplementation while 12 g produced the greatest benefit. Breads high in AX also appear to control blood glucose and insulin in adults with an already impaired glucose tolerance [60]. Fasting blood glucose, postprandial blood glucose and insulin were all significantly lower when adults with type 2 diabetes were supplemented with 15 g/d of an AX rich fiber. The mode of action behind AX on improving glucose tolerance is unknown. However, it is thought to be due

to the high viscosity of the fiber inside the lumen of the GI tract, thereby slowing the rate of glucose absorption.

The lower glycemic index of AX may also play a role. Breads made with a flour rich in AX have a relatively low glycemic index of around 59. Whole wheat flour, although high in fiber, has a glycemic index of around 99 [59]. Arabinoxylan rich bread has a similar glycemic index to that of whole grain bread but offers some distinct advantages such as improved mouth feel and tenderness. There was no significant difference in the sensory analysis between the control and a bread containing 14% AX rich fiber [59].

Inulin

Inulin is a polymer of fructose monomers and is present in such foods as onions, garlic, wheat, artichokes and bananas and is used to improve taste and mouthfeel in certain applications. It is also used as a functional food ingredient due to its nutritional properties. Because of this, inulin products can be used as a replacement for fat or soluble carbohydrates without affecting the taste and texture and still contribute to a foods nutritional value.

Enzymatic hydrolyses in the small intestine is minimal (<10%) since inulin consists of beta bonds. Therefore, it enters the large intestine and is almost completely metabolized by the microflora. When fermented they tend to favor propionate production which in turns decreases the acetate to propionate ratio leading to decreased total serum cholesterol and LDL [14], which are important risk factors for CHD.

Inulin has also demonstrated the ability to contribute to the health of the human large intestine as a prebiotic [61]. They demonstrated that inulin stimulated the growth of *bifidobacteria* while restricting the growth of potential pathogenic bacteria such as *E. Coli*, *Salmonella*, and *Listeria*. This could prove to be beneficial in such disorders as ulcerative colitis

and *C. difficile* infections. Rafter *et al.* [62] agreed with these findings and suggested they were the underlying mechanisms behind the observation that inulin decreased biological compounds associated with colonic cancer including reduced colorectal cell proliferation and water induced necrosis, decreased exposure to genotoxins and decrease interleukin-2 release.

Increased mineral absorption may also contribute to the functionality of inulin. Increased calcium absorption, by approximately 20%, was reported in adolescent girls supplemented with inulin [63]. Results from Abrams *et al.* [64], support these findings in a longer (one year) study of pubertal boys and girls consuming an inulin supplement. Subjects in the treatment group also experienced increased bone mineral density when compared to the control. The mechanisms behind these findings are still unclear but may be due to increased calcium absorption from the colon or possibly an increased solubility in the lumen of the GI tract due to short chain fatty acids. Finally, it may increase absorption through an enhancement of vitamin D.

Inulin may also provide a way to prevent and treat obesity. Cani *et al.* [65] demonstrated that oligofructose, a subgroup of inulin, increased satiety in adults which led to a decrease in total energy intake. This is thought to be due to short chain fatty acids and their ability to increase appetite suppressing hormones such as glucagon-like peptide 1 (GLP-1).

β- glucan

β-glucan is a linear polysaccharide of glucose monomers with β(1→4) and β(1→3) linkages and found in the endosperm of cereal grains, primarily barley and oats. β-glucan concentrations in North American oat cultivars range from 3.9% to 6.8% [66]. β-glucan is water soluble and highly viscous at low concentrations [67].

The physiological benefits due to β-glucan seem to stem from their effect on lipid metabolism and postprandial glucose metabolism. Many studies agree an inverse relationship

exists between consumption levels of β-glucan and cholesterol levels. Several recent studies, in both hypercholesterolemic [68] and healthy [69] subjects, found that the daily consumption of 5 g of β-glucan significantly decreased serum total and LDL cholesterol. Davidson *et al.* [36] found that only a daily consumption of 3.6 g β-glucan was needed to produce the same significant effects. The same relationship also has been reported to occur between β-glucan and postprandial glucose and insulin responses in both diabetic and healthy subjects. Biorklund *et al.* [70] found that 5 g of β-glucan from oats significantly decreased postprandial glucose and insulin levels in healthy adults. Tappy *et al.* [71] reported the same results in adult subjects diagnosed with type 2 diabetes who consumed 4.0, 6.0 or 8.4 g of β-glucan.

Most authors agree that β-glucan's viscosity in the GI tract is the most probable mechanism in which it decreases serum cholesterol levels as well as improves post prandial glucose metabolism. This gellation property may decrease bile acid absorption by increasing intestinal viscosity and increase bile acid excretion. This subsequently results in a higher hepatic cholesterol synthesis because of the higher need for bile acid synthesis [72]. The same viscosity may also delay glucose absorption into the blood thus lowering post prandial glucose and insulin levels. Nazare *et al.* [73] observed that 5 g of oat β-glucan added to an oat concentrate cereal significantly delayed, but did not reduce total glucose absorption.

The production of short chain fatty acids from β-glucan may also be a probable mechanism behind its observed metabolic effects. Fermentation of oat β-glucan has been shown to yield larger amounts of propionate [74,75]. Propionate has been shown to significantly inhibit cholesterol synthesis in humans [14] and is thought to be due to the inhibition of the rate limiting enzyme HMG CoA reductase [76].

Not all research however, agrees that β-glucan can affect lipid and glucose absorption/metabolism. Keogh *et al.* [77] observed that treatments of 8.1 to 11.9 g/d of barley β-glucan had no effect on total or LDL cholesterol in mildly hyperlipidemic adults. Cugent-Anceau *et al.* [78] not only observed that 3.5 g of oat β-glucan added to soup did not alter serum lipid profiles, but also produced no change in postprandial glucose levels.

The inconsistency between studies is thought to be due to the molecular weight (MW) and solubility of the β-glucan. The MW can be changed by several factors including food processing and the source of the β-glucan. Suortti *et al.* [79] states that heating, such as in extrusion and baking, decreases the molecular weight of β-glucan therefore decreasing its viscosity inside the GI tract. Theuwissen and Meinsk [68] and Naumann *et al.* [69] both used a β-glucan obtained through a dry milling process in which it is not significantly degraded. Keogh *et al.* [77] however, obtained their β-glucan from a hot water extraction process which may have decreased MW and in turn intestinal viscosity. Kerckhoffs *et al.* [80] supports this theory as they observed that β-glucan, when added to bread or cookies, produced no change in the lipoprotein profile of mildly hypercholesterolemic adults. However, when the same β-glucan was added to orange juice, serum LDL decreased significantly. The bread baking process decreased the MW of the β-glucan. Unfortunately, this study failed to list the MW weight of the β-glucan in the orange juice.

Different sources of β-glucan may also differ in their molecular weight and viscosity. Oats were the β-glucan source for the Theuwissen and Meinsk [68]and Naumann *et al.* [69] studies while Keogh *et al.* [77] utilized barley. Biorklund *et al.* [70] found similar results in that 5 g of β-glucan derived from oats significantly lowered serum cholesterol and postprandial glucose and insulin levels while the same level of β-glucan derived from barley produced no

effects. Since the MW can vary among oat varieties [81] it may be safe to assume that the MW can also vary among cereal grain types.

Oat and barley β-glucan also seem to differ in their solubility which would have a direct effect on intestinal viscosity. Gaidosova *et al.* [82] found that the solubility of barely β-glucan was significantly higher than that of oats.

Oat and barley varieties may also play a role on the MW of β-glucan. Yao *et al.* [81] observed large differences in viscosity between β-glucan solutions from different oat varieties as a result of β-glucan's wide range of molecular weights. Torronen *et al.* [83] using a lower MW (370,000 da) β-glucan found no changes in serum lipid profiles in men with mild to moderate hypercholesterolaemia when compared to a control. Cugent-Anceau *et al.* [84], also using a low molecular weight (80,000 da) β-glucan, found similar negative results. However, when a high MW (1,200,000 da) β-glucan was used it reduced serum cholesterol levels in the same class of subjects [85]. Kim *et al.* [74] disagreed with these findings when they reported that a lower molecular weight β-glucan bound significantly more bile acid *in vitro*. It should be noted however, that the β-glucan in this study was not in its natural form. Extracted β-glucan was treated with lichenase to yield the different molecular weights.

Pectin

Pectin is a linear polymer of galacturonic acid connected with α (1→4) bonds. Regions of this backbone are substituted with α (1→2) rhamnopyranose units from which side chains of neutral sugars such as galactose, mannose, glucose and xylose occur. Pectin is a water soluble polysaccharide that bypasses enzymatic digestion of the small intestine but is easily degraded by the microflora of the colon. Citrus fruit contains anywhere from 0.5% to 3.5% pectin with a large

concentration located in the peel. Commercially extracted pectins are also available and are typically used in food applications which require a gelling or a thickening agent.

Inside the GI tract, pectin maintains this ability to form a gel or thicken a solution. This is thought to be the likely mechanism behind its many beneficial effects on health including dumping syndrome [86], improved cholesterol and lipid metabolism [87], and diabetes prevention and control [88]. However, pectin also contains some unique abilities that may treat or prevent other diseases/disorders such as intestinal infections, atherosclerosis, cancer and obesity.

Several recent clinical studies, Rabbani *et al.* [89] and Triplehorn and Millard [90], demonstrated that oral pectin supplementation to children and infants reduced acute intestinal infections and significantly slowed diarrhea. This is thought to be due to a reduction in pathogenic bacteria such as *Shigella*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Proteus* and *Citrobacter*. This is supported by Olano-Martin *et al.* [91] who observed that pectin stimulated the growth of certain strains of *Bifidobacteria* and *Lactobacillus in vitro*. These bacteria are considered to be directly related to the health of the large intestine and their concentrations depict a healthy microflora population.

The quality of fibrin is thought to be an important risk factor for atherosclerosis, stroke and coronary heart disease. Pectin has been shown to increase fibrin permeability and decrease fibrin tensile strength in hyperlipidaemic men [92]. Although the mechanism behind this unknown it is thought to be due in part to acetate production. Pectin yields predominantly acetate in the colon which is thought to enter peripheral circulation and alter fibrin architecture.

Pectin may also have a potential role in the complicated area of cancer prevention. Nangia-Makker *et al.* [93] found that pectin was able to bind to and decrease tumor growth and

cancerous cell migration in rats fed modified citrus pectin. This is thought to be a result of pectin binding to galectin-3 and inhibiting some of its functions.

Bran

Bran is the outer most layer of a cereal grain and consists of the nucellar epidermis, seed coat, pericarp and aleurone (Figure 1). The aleurone consists of heavy walled, cube shaped cells which are composed primarily of cellulose. It is low in starch and high in minerals, protein, and fat. However, due to its thick cellulosic walls, these nutrients are virtually unavailable for digestion in monogastric species. The AACC defines oat bran as "*the food which is produced by grinding clean oat groats or rolled oats and separating the resulting oat flour by sieving bolting, and/or other suitable means into fractions such that the oat bran fraction is not more than 50% of the original starting material and has a total betaglucan content of at least 5.5% (dry-weight basis) and a total dietary fiber content of at least 16.0% (dry-weight basis), and such that at least one-third of the total dietary fiber is soluble fiber."* [94].

Bran from a wide array of cereal grains have been shown to have an effect on postprandial glucose levels, serum cholesterol, colon cancer, and body mass. Although the efficacy of bran may change due to its source, the purpose of this section will just evaluate bran's general effect on the parameters listed above.

In a recent study of healthy adults, 31 g of rye bran decreased peak postprandial glucose levels by 35% when compared to the control [95]. This effect may be due to the high AX content in rye bran. Arabinoxylan, as discussed previously, may increase intestinal viscosity and slow nutrient absorption. In a more lengthy study, Qureshi *et al.* [96] found that subjects suffering type 1 and 2 diabetes decreased their fasting glucose levels due to the daily consumption of 10g of stabilized rice bran over 2 months. The results may arise due to an increased intestinal

viscosity but is more likely a result of a decreased carbohydrate/caloric intake. Koh-Banerjee *et al.* [32], in a larger clinical study, supports this theory in their finding that for every 20 g/d increase in consumption of bran body weight decreased by 0.80 lbs. It should be noted that this data remained significant even after adjustment for fat and protein intake, daily activity, caloric intake and baseline weight. In an earlier study, Zhang *et al.* [97] observed that adults with ileostomies, consuming bread rich in rye bran, significantly increased the ileal excretion of fat, nitrogen and energy. This study suggests bran did not delay nutrient absorption in the small intestine but hindered it.

In addition to a possible effect on carbohydrate absorption and metabolism, bran also seems to have the same effect on lipids. In a long term clinical study, Jensen *et al.* [98] reported that an increased daily consumption of bran significantly decreased the risk of coronary heart disease in healthy adult men. This is most likely due to the data reported by Qureshi *et al.* [96] who found that 10 g of rice bran consumed for 8 weeks was able to decrease serum total cholesterol, LDL cholesterol and triglycerides. The mechanisms behind these effects may be two fold. The reduction in cholesterol levels is likely due to an increase in bile acid synthesis. Andersson *et al.* [99] found that oat bran doubled the serum concentration of 7α-hydroxy-4-cholesten-3-one (α-HC), which is a metabolite in the synthesis of bile acids that is oxidized from 7α-hydroxycholesterol. The reduction in serum triglyceride levels may be a result of a decreased absorption of fat from the small intestine [97].

Cellulose

Cellulose is a linear chain of β(1→4) linked glucose monomers and is the structural component of cell walls in green plants and vegetables. It is water insoluble and inert to digestive

enzymes in the small intestine. However, it can go through microbial fermentation to a certain degree in the large intestine in turn producing SCFA.

Natural cellulose can be divided into two groups: crystalline and amorphous. The crystalline component, which is made up of intra and intermolecular non covalent hydrogen bonds, make cellulose insoluble in water. However, many modified celluloses such as powdered cellulose, microcrystalline cellulose and hydroxypropylmethyl cellulose have been developed and are used as food ingredients. The difference between natural and modified celluloses is the extent of crystallization and hydrogen bonding. When these hydrogen bonds are disrupted and the crystallinity is lost, the cellulose derivative becomes water soluble [100].

Little research has been conducted evaluating the effects of cellulose in humans. Therefore, studies in other models such as the rat will be discussed. The translation to human relevance is poorly understood and debatable. Cellulose pills have been made available for human consumption with the theory that cellulose may decrease a person's caloric intake. Although no human studies could be found to support this several animal studies using cats [101], dogs [102] and rats [103] have shown that increasing dietary cellulose can reduce daily energy intake. This is most likely a dilution factor since cellulose is virtually undigested in the small intestine and only 51% metabolized by the microflora of the colon.

Many studies have evaluated the effect of cellulose on blood glucose and insulin levels in many different models. However, the data is extremely contradictory and may depend on the subject, type of cellulose and other unknown factors. Using the rat [104], dog [105] and cat [106], natural cellulose was shown to decrease postprandial glucose and insulin levels. However, similar studies in pigs [107] and humans [108] demonstrated that natural cellulose had no effect on these parameters. Studies using modified celluloses showed more consistent data.

Microcrystalline cellulose has shown the ability to decrease blood glucose levels in the pig [109] and rat [110]. Complimenting this, methylcellulose had demonstrated the same effects in humans. Lightowler and Henry [111] found that adding only 1% high viscosity hydroxypropylmethylcellulose (HV-HPMC) to mashed potatoes decreased postprandial glucose levels by 37% in healthy adults. Also, Maki *et al.* [112] reported an acute 35 % reduction in postprandial blood glucose due to 4 g of HV-HPMC in overweight subjects.

Modified cellulose has also been reported to effect lipid metabolism. Maki *et al.* [113,114] both observed a significant reduction in total and LDL cholesterol in hypercholesterolemic adults consuming 5 g/d of HV-HPMC for four weeks. Interestingly, in subjects already receiving statin drugs, HV-HPMC was able to further reduce total and LDL cholesterol.

According to this, modified celluloses may be more beneficial than that natural cellulose. These modified celluloses, as described above, act like soluble fiber thus adding to the viscosity of the GI tract. Therefore, it is assumed that increased intestinal viscosity delays nutrient absorption and increases bile acid excretion.

Resistant Starch

Resistant starches (RS) are defined as any starch not digested in the small intestine [115] and is classified into four basic “types”. Type 1 (RS1) is made up of starch granules surrounded by an indigestible plant matrix. Type 2 (RS2) occurs in its natural form such as in an uncooked potato and high amylose maize. Type 3 (RS3) are crystallized starches made by unique cooking and cooling processes. Type 4 (RS4) is a starch chemically modified by esterification, crosslinking, or transglycosylation and is not found in nature. Few studies have compared types,

but one recent study by Haub *et al.* [116] reported that cross-linked RS4 elicited a greater glucose lowering effect than the more commonly tested RS2.

A majority of human studies involving RS have shown a decrease in postprandial blood glucose and insulin levels. However, it is difficult to completely understand these effects due to differences in study design and the type of RS used. Behall *et al.* [117] found that women consuming 0.71 g, 2.57 g or 5.06 g of RS had significantly lower postprandial glucose and insulin levels when compared to the control. However, this study failed to maintain an equal amount of available carbohydrate between the treatments and control. Therefore, it is difficult to determine whether the attenuation of glucose and insulin was due to the RS or the fact that there was less available carbohydrate in the meal. Similarly, Reader *et al.* [118] reported that 7.25 g of RS added to an energy bar decreased blood glucose and insulin levels in healthy adults. But, ingredients, amount of ingredients and nutrient levels were different for each treatment. A recent study by Al-Tamimi *et al.* [119], however removed these variables by controlling for non starch ingredients and available carbohydrates. It was reported that postprandial blood glucose and insulin levels were significantly reduced with the supplementation of 30 g of RS4.

Several studies report that longer term consumption of a RS may decrease fasting cholesterol and triglyceride levels. In a 5 week study, Behall *et al.* [120] found that men consuming 34% of their energy from high amylose maize, when compared to a high amylopectin carbohydrate, had significantly reduced fasting cholesterol and triglyceride levels. Resier *et al.* [121] reported similar results in an isocaloric and isonutrient diet with either high amylose maize or fructose. Porikos and Van Itallie [122] suggest that an interaction exists between sucrose, and therefore most likely fructose, and saturated fatty acids in turn promoting serum triglyceride levels. Interestingly, the relationship does not seem to exist for polyunsaturated fatty acids. The

likely mechanism behind the ability of RS to decrease cholesterol levels is an increased intestinal viscosity [127], However, some studies, such as Jenkins *et al.* [123], report conflicting data as RS2 and RS3 had no effect on serum lipid profiles. While using the same type of RS, subjects were only tested for two weeks. It may be that the RS requires a longer period of time to promote an effect.

Research has also been conducted which evaluates the effect of RS on fat oxidation and storage. However, data between studies are contradictory with no clear conclusions. Tagliabue *et al.* [124] reported that RS2, obtained from raw potatoes, was able to increase fat oxidation 5 h postprandial. However, the test diet, consisting of the RS2, had significantly less gross and metabolizable energy. Therefore, it is difficult to determine if the increase fat oxidation was due to the RS2 or a decreased caloric intake. A 10 week study by Howe *et al.* [125] may suggest the later. High amylose starch, compared to a high amylopectin, produced no change on fat oxidation when an isocaloric diet was consumed. Conversely, Robertson *et al.* [126] reported that 30 g of RS2 added to healthy subjects habitual diet resulted in a significant decrease in subcutaneous abdominal adipose tissue non-esterfied fatty acid (NEFA) and glycerol release. This may be a result of increased peripheral SCFA metabolism or ghrelin secretions.

Conclusions

In a simplified definition, dietary fiber is a carbohydrate that resists digestion and absorption and may or may not undergo microbial fermentation in the large intestine. This definition is essentially the basis to its correlation between consumption levels and possible health benefits. Dietary fiber consists of many different constituents however; some are of particular interest and include arabinoxylan, inulin, β-glucan, pectin, bran and resistant starches. These individual components of dietary fiber have been shown to significantly play an important

role in improving human health. Current research is paying particular attention to these elements; although further research is needed to better understand particular health claims and the mechanisms involved.

A large amount of research has reported an inverse relationship between fiber consumption and the risk for coronary heart disease and several types of cancer. For that reason, the FDA as adopted and published the claim that increased consumption of dietary fiber can reduce the prevalence of coronary heart diseases and cancer. The mechanisms behind these findings are still unclear. However, it is thought to be attributed to several factors including increasing bile acid excretion, decreased caloric intake, increased short chain fatty acid production, carcinogen binding effects, increased antioxidants, and increased vitamins and minerals.

Although not adopted by the FDA as of yet, dietary fiber is suggested to play a role in other conditions such as obesity and diabetes. Although some data are contradictory, a majority of the studies regarding dietary fiber report a decrease of these two conditions with increased consumption of fiber.

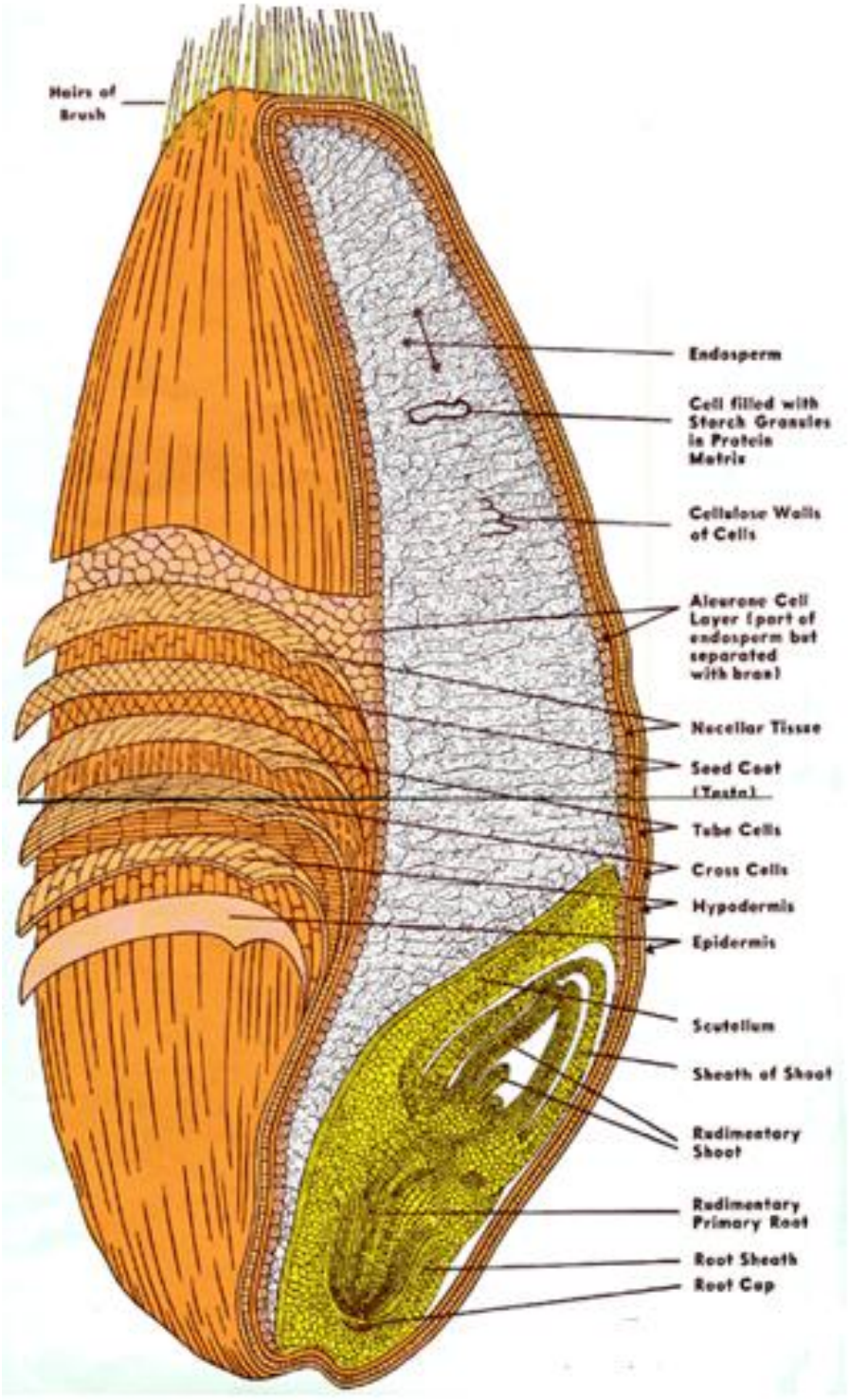
The digestive and viscosity characteristics of dietary fiber are the likely modes of action which affects diabetes and obesity risk. These mechanisms appear to decrease nutrient absorption therefore, decreasing metabolizable energy. Dietary fiber may also be able to decrease gross energy of a food due to its lower energy density.

Further studies are needed in certain areas of dietary fiber research. Those of particular interest are in the components of fiber such as β-glucan, arabinoxylan, resistant starches, *etc.* These sub fractions may give a better understanding of the health benefits of dietary fiber as well as the mechanisms behind them.

Table 1.1 Constituents of dietary fiber according to the America Association of Cereal Chemists' (23)

Non Starch polysaccharides & oligosaccharides
Cellulose
Hemicellulose
Arabinoxylans
Arabinogalactans
Polyfructoses
Inulin
Oligofructans
Galacto-oligosaccharides
Gums
Mucilages
Pectins
Analagous carbohydrates
Indigestible dextrins
Resistant maltodextrins
Resistant potato dextrins
Synthesized carbohydrates compounds
Polydextrose
Methyl cellulose
Hydroxypropylmethyl cellulose
Resistant starches
Lignin substances associated with the NSP and lignin complex
Waxes
Phytate
Cutin
Saponins
Suberin
Tannin

Figure 1.1. Longitudinal and cross sections of a wheat kernel (From: www.namamillers.org/images/Kernelofwheat.gif).



Chapter 2 - Effect of dietary induced elevations of short chain fatty acids on hepatic and myocellylar lipid metabolism in gilts

Introduction

The prevalence of type II diabetes mellitus (T2DM) and obesity has significantly increased over the past several decades (Mokdad et al., 2003) and these conditions have been linked to metabolic syndrome (MS) (Petersen et al., 2007). According to Grundy et al. (2004), more than 50 million Americans are afflicted with MS and has been directly linked to the high mortality rates of heart disease and diabetes (Murphy et al., 2012).

For prevention and treatment, the most widely promoted and accepted method is proper energy balance and energy restriction (Cummings et al., 2002; Krauss et al., 2000a). Earlier work by Laakso et al. (1988) found that a hypocaloric diet (500 kcal/d) improved glucose disposal rates (GDP) by 71% in obese non-insulin diabetic subjects. Interestingly, similar subjects placed on a eucaloric diet and treated with exogenous insulin only had a 17% improvement in GDP. Hypocaloric diets also decrease adiposity in overweight and obese subjects. Gardner et al. (2007) found that 4 different hypocaloric diets (Atkins, Zone, LEARN and Ornish), which differed in their compositions of carbohydrate, fat and protein, resulted in significant reductions in total body adiposity after 2, 6 and 12 months.

Unfortunately, the ability to maintain a proper energy balance for an extended period of time is difficult for most people. Therefore, researchers have evaluated alternative methods independent to that of calorie counting and restriction. One such method is increasing the daily consumption of dietary fiber (DF). Many epidemiological studies show an inverse relationship between DF intake and diabetes (de Munter et al., 2007; Meyer et al., 2000; Schulze et al., 2004)

and obesity (Du et al., 2010; Koh-Banerjee et al., 2004). Although more inconsistent, clinical data has also shown the ability of DF to improve glucose/insulin kinetics (Pereira et al., 2002; Robertson et al., 2005; Weickert et al., 2006) and regulate body weight (Birketvedt et al., 2000; Rigaud et al., 1990; Solum et al., 1987).

The means by which DF is able to regulate a subject's glycemic response and/or adiposity remains unclear and several potential mechanisms have been postulated. Lattimer and Haub (2010) suggest it is likely due to energy restriction through DF's ability to decrease the metabolizable energy (ME) of the diet. This theory is supported in an earlier study by Baer et al., (1997) which found that increasing levels of DF decreased the ME of mixed diets in healthy adults. Other authors, however, propose that increased plasma concentrations of short chain fatty acids (SCFA) produced from the fermentation of DF produces a peripheral effect on substrate metabolism (Robertson et al., 2005).

Robertson et al. (2005) found that adults consuming 30 g/d of soluble fiber over four weeks elicited significant increases in glucose disposal (via euglycemic-hyperinsulinemic clamp) and insulin sensitivity via an oral glucose tolerance test independent to that of weight loss. The authors postulated that the enhanced glycemic control may be due to the enhanced uptake and oxidation of SCFA by skeletal muscle. Brighenti et al. (2006), using a second meal effect design, also concluded that SCFA may be responsible for the observed improvement in glucose tolerance.

Excluding pharmacological intervention, it is generally accepted that insulin resistance is improved the best through mechanisms (e.g. weight loss) that lead to increased β-oxidation, which has been detailed by Kelley and Mandarino (2000). However, the data reported by Robertson et al. (2005) provide theoretical evidence that DF may improve peripheral substrate

metabolism independent to that of weight loss. Although, the mechanisms are unclear, several authors suggest it may be a result of increased SCFA plasma concentrations (Brighenti et al., 2006; Tarini and Wolever, 2010). Similarly, results from Gao et al. (2009) found that obese mice supplemented with sodium butyrate had improved insulin sensitivity through enhanced thermogenesis and fatty acid oxidation which was supported by significant increases in skeletal muscle expression of peroxisome proliferator-activated receptor gama (PGC-1α) and carnitine palmitoyltransferase 1 (CPT1) mRNA. Unfortunately, Gao et al. (2009) failed to control for energy intake; therefore, it is unknown if the observed changes in insulin sensitivity and gene expression were due to the SCFA butyrate or simply a result of energy restriction.

Therefore, this study will be the first to compare the effects of both energy restriction and fermentable DF on body composition factors (body weight, adiposity) and markers of metabolic health (plasma glucose, insulin, and lipids). Furthermore, the expression and activity of several key regulators of substrate metabolism including CPT1, and PGC-1α will be measured to better understand how DF may elucidate changes in β-oxidation independent to that of energy restriction.

Materials and Methods

Animals

This study was conducted in a single room at the Kansas State University Swine Research and Teaching Center and was approved by the Kansas State University Institutional Animal Care and Use Committee. At approximately 36.4 kg, 150 crossbred gilts were moved to group pens (1.8 x 3.6 m) with 6 gilts per pen. Pens were equipped with a single feeder and nipple waterer to allow ad libitum access to feed and water. Pigs were offered a standard finisher

diet of corn and soybean meal for 40 d and then individually weighed. 18 gilts, averaging 72.2 ± 0.68 kg, were then selected based on homogeneity of weight and moved into individual pens (1.8 x 1.8 m). Each pen was equipped with an individual feeder and nipple waterer.

Diets

A 7 d run-in period whereby all gilts were offered free access to the control diet was utilized to adapt the pigs to individual housing and feeding. Following the run-in period, pigs were randomly assigned to one of three diets (Table 2.1): control (CON), high fiber (HF) and energy restricted (ER). All diets were matched for protein, fat, lysine, Ca and P. The CON and HF diet were offered at ad libitum. Gilts consuming the ER diet were pair fed to the gilts fed the HF diet matched for total body weight gain. During the first week, metabolizable energy (ME) intakes were matched between the treatment diets. Thereafter, body weights were measured weekly and intake was adjusted accordingly to ensure similar growth rates to minimize metabolic effects due to differences in growth rate.

Growth performance

Feed intake was recorded weekly for individual pigs consuming the CON diet, while feed intake was recorded daily for individual pigs consuming the HF and ER diets. Feed amounts for gilts consuming the ER diet were adjusted daily to match the ME intake of gilts consuming the HF diet. Body weight was measured initially and then assessed weekly. Body fatness was estimated by measuring back fat thickness via ultrasound (Aloka 500, 5 mHz linear array transducer, Wallingford, CT) at the 14^{th} rib.

Sample collection

On days 0 and 42, pigs were fasted for 10 h and then sedated with an IM injection of acepromazine (0.3 mg/kg), xylazine (0.4 mg/kg) and ketamine (15 mg/kg). Once in lateral recumbancy, they were transferred to a procedure table. Approximately 14 ml of blood was collected from the jugular vein into 2 tubes containing sodium floride and potassium oxalate as glycolytic inhibitors (BD Vacutainer, Franklin Lakes, NJ). Blood samples were centrifuged at 2000 x g for 10 min after collection. Plasma was then separated and frozen at -80°C for later analysis of glucose, insulin, NEFA, TG, acetate, propionate and butyrate.

Liver samples were obtained by modified methods of Heo et al. (Heo et al., 2005) and Washburn et al. (Washburn et al., 2005). Briefly, the entry area was clipped, surgically prepared with betadine scrub and isopropyl alcohol (70%) and anaesthetized by local infiltration of 2 ml lidocain hydrochloride (2%). A small incision (< 5mm) was made at the 10^{th} and 11^{th} intercostals space, 8 cm ventral to the ventral aspect of the 10^{th} rib. A 14 gauge spring loaded biopsy needle (SABD-1409-10-T, US Biopsy, Franklin, IN,USA) was inserted caudal-cranially toward the liver. Ultrasound was used to guide the needle into the liver and avoid puncture of other vital organs. Approximately 200 mg of liver tissue was snap frozen in liquid nitrogen and another 200 mg of tissue was placed into an RNA storage solution (RNAlater, AM7023, Applied Biosystems, Austin, TX, USA) and stored at -20°C. Muscle samples were taken by modified methods of Ville et al. (1992) and Haub et al. (1999). Briefly, a small incision was made 5 cm distal and 5 cm caudal to the greater trochanter with a surgical blade (#15 Bard Parker, Fisher Scientific Inc.). A 5 mm Bergstrom biopsy needle (11420-06 Dixons Surgical Instruments Ltd, Wickford, UK) was inserted distally perpendicular to the femur at 45° to a depth of 5 cm. Approximately 200 mg of muscle tissue was snap frozen in liquid nitrogen and another 200 mg

was placed into an RNA storage solution. Following the procedures, pigs were monitored twice daily for localized infections at the incision sites. No infections occurred.

Plasma analyses

All biochemical analyses were run in duplicate. Plasma was analyzed for glucose by an automated glucose analyzer (YSI 2300 STAT Plus, YSI Life Sciences, Yellow Springs, OH), insulin by a porcine specific ELISA (#80-INSPO-E01, ALPCO Diagnostics, Salem, NH), TG by enzymatic ELISA (#10010303, Cayman Chemical Co., Ann Arbor, MI), NEFA by enzymatic colormetric procedure (NEFA-HR, Wako Chemical USA, Richmond, VA).

Plasma SCFA (acetate, propionate and butyrate) concentrations were determined as previously described (Brighenti, 1997) using gas chromatography with a fused-silica FFA capillary column (Hewlett Packard HP-FFAP 30m x 0.53 mm i.d., 1µm phase film thickness) in a flame ionization detector (Hewlett Packard 5890, Sampler 7673A automatic injector). Briefly, plasma proteins were precipitated with 16% metaphosphoric acid for 30 min at 60°C. Samples were then centrifuged at 8000 x g for 30 min. The clear supernatant was recovered and 1µL was injected in a splitless fashion onto the FFAP column.

RNA isolation and real-time PCR analysis

The quantity of hepatic and myocellular mRNA for PGC-1α, CPT1α, CPT1β and 18S was determined by RT-PCR (Mullins et al., 2012). RNA was isolated from muscle and liver samples using a commercial kit (RNeasy Lipid Tissue Mini Kit, Qiagen, Valencia, CA) and quantified via spectroscopy (Nanodrop-100, Nanodrop Technologies Inc., Wilmington, DE). High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) was used to synthesize coding DNA from 2µg of total RNA. Samples were analyzed in duplicate by quantitative RT-PCR with 1/20 of the cDNA product in the presence of 200 n*M* gene-specific

forward and reverse primers (Table 2.2) using SYBR green fluorescent detection (ABI 7599 Fast, Applied Biosystems). Delta delta Ct technique was used to quantify mRNA abundance. 18S was used to normalize values and abundance within liver and muscle tissue did no differ in response to diet, making it a valid reference gene. Relative expression is expressed as fold changes of mRNA abundance to day 0 for each respective group.

Statistical analyses

One gilt in the CON group became sick during the third week of the study and was excluded from statistical analysis. Physical and metabolic data were analyzed as a completely randomized design with pig as the experimental unit. An ANOVA was performed using the GLM procedure of SAS (Cary, NC) with the fixed effect of diet (CON, HF and ER). Least squares means were computed and preplanned orthogonal contrasts were used to analyze the effect of diet. Data are reported as least squares means ± SEM. Statistical significance was declared at $P \leq 0.05$.

The Brown and Forsythe test (Brown and Forsythe, 1974) was utilized to determine homogeneity of variance. Variances for CPT-1α, CPT1β, PGC1α and 18s values failed to meet the assumption of constant variance and were transformed using the natural logs of mRNA expression values. This transformation corrected the assumption violation.

Results

The mean physical and plasma response variables on day 0 and 42 are listed in Table 2.3. Figures 2.1 – 2.8 illustrate the mean change from day 0 to 42 with statistical contrasts testing the effect of diet.

Weight and body composition

Average daily gain (kg of BW gain/d, ADG) was lower ($P < 0.05$) for HF and ER when compared to the CON (Figure 2.1). However, there was no difference ($P = 0.81$) for ADG between HF and ER. When compared to CON, HF and ER resulted in a lower ($P = 0.0009$) back fat thickness (Figure 2.2). Additionally, gilts consuming the HF diet had a lower ($P = 0.018$) back fat thickness when compared to those consuming the ER diet.

Energy intake and efficiency

Average daily feed intake (kg/d, ADFI) was lower ($P <0.0001$) for the treatment diets (HF and ER) when compared to the control (Figure 2.3A). The ER gilts also consumed less ($P = 0.06$) feed per day when compared to the HF gilts. The average daily energy intake (kcal of ME/d, ADEI) was lower ($P < 0.0001$) for the treatment diets when compared to the control (Figure 2.3B). There was no difference ($P = 0.44$) of ADEI between the HR and ER diets. The treatment diets utilized metabolizble energy (kcal/kg of BW gain) more efficiently ($P = 0.02$) when compared to the control (Figure 2.4). There was no difference in energy utilization between the HF and ER diets ($P = 0.24$).

Glucose and insulin

The HF and ER gilts tended ($P = 0.07$) to have a lower fasting plasma glucose levels when compared to the control (Figure 2.5A). There was no difference ($P = .70$) in the change of fasting glucose levels between HF and ER. The HF and ER diets also tended to result in increased ($P = 0.06$) fasting plasma insulin levels when compared to the control (Figure 2.5B). There was no difference ($P = 0.52$) in the change in fasting insulin levels between the HF and ER diets.

TG and NEFA

The HF diet resulted in an increased (P = 0.0015) fasting TG concentration when compared to the CON and ER diets while no difference (P = 0.53) was observed between CON and ER diets (Figure 2.6A). The HF gilts also decreased (P = 0.018) their fasting NEFA levels when compared to the CON (Figure 2.6B). Similar to that of the TG, no differences (P = 0.64) were found in fasting NEFA concentrations between the CON and ER diets.

Short chain fatty acids

Pigs consuming the HF diet increased (P < .0001) their fasting plasma acetate concentrations when compared to those on the CON and ER diets (Figure 2.7A). There was no difference (P = 0.87) in the change of fasting plasma acetate levels between the CON and ER diets. Fasting plasma propionate concentrations increased (P = 0.0002) in the HF diet when compared to the CON and ER (Figure 2.7B). Also, the ER diet tended to have a decreased (P = 0.61) fasting plasma propionate concentration when compared to the CON. Fasting plasma butyrate concentrations increased (P = 0.0003) during the HF diet compared to the CON and ER diets (Figure 2.7C). There was no difference (P = 0.69) in the change of fasting plasma butyrate concentrations between the CON and ER diets.

Gene expression

Pigs consuming the HF diet tended to have a lower (P = 0.09) fold change of relative mRNA expression for CPT1α in the liver compared to the CON and ER diets (Figure 2.8A). No differences were found in expression of CPT1α between the CON and ER gilts (P = 0.92). Similarly, the fold change for PGC-1 α tended to me smaller (P = 0.06) for the HF group when compared to CON and ER (Figure 2.8C). There was no difference in PGC-1 α mRNA expression between the CON and ER diets (P = 0.95).

No differences were found in fold change of relative mRNA expression for CPT1β in the muscle for the HF gilts compared to CON and ER (P = 0.76, Figure 2.8B). There was also no difference in relative expression of CPT1β between the CON and ER diets (P = 0.92). Likewise, the fold change for PGC-1α was similar in the HF gilts and the CON and ER gilts (P = 0.64, Figure 2.8D). Also, no differences were found in relative mRNA expression of PGC-1α between the CON and ER diets (P = 0.10).

Discussion

The major objective of this study was to determine if a diet high in DF, and the subsequent production of SCFA, altered body composition and lipid metabolism independent to that of energy restriction. Interestingly, the HF diet significantly altered both body adiposity and serum lipids independent to that of simple energy restriction.

Gilts consuming a diet high in DF decreased their fasting plasma NEFA concentrations

The HF diet elicited a twofold decrease in fasting NEFA concentrations when compared to both CON and ER. The effect of DF on NEFA concentrations observed in the present study is supported by many human in vivo studies which found that meals higher in DF resulted in a decrease in NEFA concentrations both postprandially (Robertson et al., 2005; Tarini and Wolever, 2010) and in the second meal effect (Brighenti et al., 2006). Furthermore, in vitro and in vivo data demonstrate that SCFA are a controlling factor in the process. Previous work by Wolever et al., (1989) and Akanji et al. (1989) found that serum NEFA values decreased in adults receiving rectal and intravenous infusions of acetate, respectively, suggesting that acetate inhibits lipolysis. In fact, Hong et al. (2005), using mouse adipocytes in vitro, reported that

acetate and propionate were antilipolytic with effects similar to that of insulin. The mechanism is likely a result of G protein-couple receptor (GPCR) stimulation and subsequent inhibition of hormone sensitive lipase (HSL). Furthermore, PGC-1α activates protein kinase A (PKA) in turn activating HSL (Lelliott and Vidal-Puig, 2004). Therefore, the tendency for PGC-1α to have a lower fold change in the HF gilts of this study provides additional support for lipolytic inhibition due to the present HF treatment.

The significantly higher fasting plasma concentrations of all three SCFA in the HF gilts support previous work in sows (Serena et al., 2009). The most pronounced change occurred for acetate where fasting concentrations increased an average of 115.94 ± 12.42 μmol/L over the course of the study for the HF diet. Plasma concentrations of propionate and butyrate were similar to those previously reported in growing pigs and sows consuming a high fiber diet while acetate values are somewhat lower which may be due to the different fiber sources used between studies (Serena et al., 2009; Yen et al., 2004). Although human studies evaluating the plasma concentrations of SCFA in humans are limited, Robertson et al. (2005) reported fasting plasma acetate and propionate concentrations of 217 ± 18.2 μmol/L and 9.09 ± 2.48 μmol/L, respectively, in healthy adults. Their values are similar to the fasting values 259.03 ± 11.21 μmol/L and 9.33 ± μmol/L observed in the HF gilts of this study.

Gilts consuming a diet high in DF did not improve their fasting glucose and insulin concentrations

A decrease in circulating NEFA concentrations has been suggested to improve glucose and insulin kinetics both postprandially (Robertson et al., 2005; Tarini and Wolever, 2010) and in a second meal effect (Brighenti et al., 2006). Certainly, a decrease in NEFAs may improve glucose disposal rates according to the glucose fatty-acid cycle (Randle, 1981); however, this

theory was not supported in the current study. Fasting plasma glucose levels tended to decrease (P = 0.07) in both treatment diets when compared to the control. Therefore, these data do not support the fatty-acid cycle theory since NEFA concentrations did not decrease in the ER gilts. Others (Fernandes et al., 2011; Tarini and Wolever, 2010) have supported the theory that SCFA in an acute setting (postprandially) improve glucose tolerance thereby decreasing the risk for type II diabetes. Conversely, the current data show no improvement in glucose handling due chronic daily elevations in SCFA. Similarly, other chronic studies in pigs (Weber et al., 2010) and humans (Jenkins et al., 2002; Juntunen et al., 2003; Robertson et al., 2005; Ventura et al., 2009) found no effect of DF supplementation on fasting glucose and insulin values. Admittedly, we did not measure postprandial glucose concentrations or insulin sensitivity; however, improvements in glucose kinetics should have resulted in decreased fasting concentrations. Finally, although there was a trend for fasting glucose to decrease with energy restriction, the change was small indicating that its physiological significance is likely negligible. Insulin concentrations tended to be reduced (P = 0.06) in the CON pigs. Although this is contradictory to the theory that energy restriction improves fasting insulin concentrations, the difference in values are slight and likely to be physiologically irrelevant.

Gilts consuming a diet high in DF increased their fasting plasma TG concentrations

Gilts consuming the HF diet increased their fasting TG concentrations by 56%, which is somewhat similar to the 37% increase observed by Weber et al. (2010) in growing gilts fed a high fiber diet. An increase in circulating TG levels has also been observed in adults fed a diet higher in DF (Jenkins et al., 1991; Wolever et al., 2002). Evidence suggests these elevations may be a result of increased levels of SCFA as rectal and intravenous infusion of acetate have been shown to raise serum TG concentrations in adults (Wolever et al., 1991; Wolever et al.,

1995; Wolever et al., 1989). Certainly, it is important to investigate the mechanisms behind these observations and determine if the elevations in TG are due to augmented lipogensis or inhibited hydrolysis of TG.

Results by Wolever et al. (1995) support a lipogenic affect of acetate whereby a significant concentration of labeled carbon atoms (^{13}C) from acetate were recovered in circulating TG after rectal infusions of acetate. Correspondingly, 25% of C^{14} from acetate was recovered in fatty acids in livers of rats fed an ad libitum diet (Lyon et al., 1952). This value rose to 50% after a single administration of 5 g of glucose. Therefore, the chronically high levels of circulating acetate in the current study may have indeed resulted in an enhanced de novo lipogenesis thus increasing TG plasma concentrations. While it is true that propionate has been shown to hinder acetate's lipogenic affect (Nishina and Freedland, 1990; Wolever et al., 1995) the 28:1 ratio of acetate to propionate in the HF group may have enough to offset this inhibition.

PGC-1α knockdown results in significant downregulation of both fatty acid oxidative genes and rates of fatty acid oxidation (Koo et al., 2004; Leone et al., 2005). Therefore, the tendency of PGC-1α to have a lower hepatic mRNA fold change in the HF gilts may likely decrease their hepatic fatty acid oxidation. This would help support the theory of increased lipid synthesis due to acetate as β-oxidation is inhibited during periods of de novo lipogenesis. It should be noted; however, that lipogenesis in pigs occurs predominately in adipocytes unlike its largely hepatic location in humans. The lower fold change of CPT1α provides further support of an inhibited hepatic β-oxidation rate in the HF gilts as CPT1α is upregulated during periods of increased fatty acid oxidation (Kelley and Mandarino, 2000). Since acetate is rapidly oxidized and converted to acetyl-CoA in the cytoplasm a subsequent accumulation of malonyl-CoA may have occurred in the present study, thus inhibiting CPT1α.

Although less supported, theoretical evidence exists that may support an antihydrolytic affect of DF and/or SCFA through the stimulation of angiopoietin-like 4 (ANGPL4). It is widely accepted that this secretory protein inhibits the activity of lipoprotein lipase (LPL) in turn increasing peripheral concentrations of TG (Zhu et al., 2012). Furthermore, it has been shown that specific gut microbiota such as *Lactobacillus Paracasei* can increase the expression of ANGPL4 (Aronsson et al., 2010). Therefore, it could be postulated that alterations in gut microbiota and/or the production of SCFA, due to increased levels of DF fermentation, increased circulating levels of ANGPL4 thereby increasing TG concentrations.

Regardless of its mechanism, DF and SCFA appear to increase TG plasma levels. This seems to contradict the recommendation that diets high in DF promote metabolic health. On the surface, it may appear that DF-induced increases in TG are "unhealthy", as TG is a biomarker used to assess CVD risk and metabolic syndrome (Grundy et al., 2004). However it is recommended by many health related associations (American Diabetes Association, American Dietetic Association and American Heart Association) to increase DF and plant fiber (e.g. whole grains) (ADA, 2000; Krauss et al., 2000b; Marlett et al., 2002). The discrepancy may be a result of the role energy intake plays in health outcomes. Thus, clinical benefits of DF might be more dependent on energy intake, which is supported by Brownlee et al. (2010). It is likely that the health benefits of DF are due to its ability to lower a diets ME and not the production of SCFA. On the other hand, the other known benefits of DF may supersede these increases in TG concentrations thus improving metabolic factors such as adiposity, glucose disposal and lipidemia. Several of the benefits include increasing gastrointestinal viscosity which may slow or prevent nutrient absorption in the small intestine (Jenkins et al., 1978; Schneeman, 1998) and increasing satiety thereby decreasing total caloric intake (Samra and Anderson, 2007).

Gilts consuming a diet high in DF reduced their energy intake, body weight and adiposity

The ADEI for the HF and ER groups were significantly lower when compared to the CON. This was a result of a reduction in the energy density of the diet due to the high DF content. This has also been observed in human diets were increasing levels of DF decreased a diets ME (Baer et al., 1997). This outcome was no unexpected given the difference between gross energy (GE) and ME. The HF gilts also had a significantly lower ADFI which also contributed to their lower ADEI. The HF gilts ate considerably less than the CON although they were both offered at ad libitum. Therefore, ADG for the treatment diets were significantly lower when compared to CON, while no differences in ADG existed between the HF and ER diets. These data are in agreement with other studies who reported decreased ADG due to the inclusion of DF (Hedemann et al., 2006; Kornegay, 1981).

Although ADG was similar between the treatment diets, change in back fat thickness was twofold higher in the ER gilts compared to those consuming the HF diet. This is interesting since there was only a small numerical difference of ADEI between the HF and ER diets. The numerically higher 474 kcal/d is approximately 8% of the HF gilts ADEI. It is difficult to compare this number to an adult's daily caloric intake as human diets are expressed as kcal of GE. Using ME is a more accurate measurement of caloric requirements and caloric content of food. However, if we estimate an adult's daily caloric intake in ME to be between 1500 and 2000 kcal/d, then this 8% decrease in caloric intake for an average adult may translate into 145 - 166 kcal/d of ME, which is speculation as the difference between GE and ME is affected by the type of food consumed.

Indeed, this 474 kcal difference in ADEI may be represented in the differences in back fat thickness. It should be noted though, that the HF gilts were 499 kcal more metabolically efficient when compared to the ER gilts, which may offset the differences observed in ADEI. However, the unequal caloric requirements for lean and adipose tissue accretion seem to limit the relevance of this theory. It is also unlikely that the difference in adiposity was due to increased fatty acid oxidation as the relative expressions of CPT1β and PGC-1α in myocytes were unchanged by diet.

Although carcass characteristics were not measured, differences in protein accretion may explain the differences in body composition between the treatment diets. Since the diets were balanced on a g/kg of diet basis and the fact that the ADFI were different between diets, the macronutrient intakes on a g/d basis were different. In fact, the ER gilts consumed approximately 276.4 g of CP per d compared to the 356.6 g/d of the HF gilts. Indeed, this may be enough to limit protein accretion and decrease the amount of lean mass tissue which may help explain the differences in adiposity with similar body weights. Visceral organ mass may also play a role as it has been demonstrated that diets higher in DF increase gastrointestinal, liver and kidney weight in pigs (Anugwa et al., 1989; Pond et al., 1988).

Gilts consuming a diet high in DF improved their energy efficiency

As eluded to previously, gilts consuming the treatment diets utilized ME more efficiently compared to those consuming CON. Previous studies in pigs and cattle concur with these findings demonstrating increases in ME efficiency due to a reduced energy intake (Barea et al., 2010; NRC, 1996). Many human studies also support this observation, as decreases in resting energy expenditure as a result of energy restriction have been reported (Schwartz and Doucet, 2010). The reduction in energetic efficiency of the CON gilts is likely due to the thermic effect

of feeding and greater body heat production (Barea et al., 2010; Silva, 2006). It should be noted that the gilts consuming the HF diet were noticeably less active during feeding and inspection periods. Although this was a subjective observation, the decreased activity may have contributed to the numerical difference of energetic efficiency between the HF and ER groups.

Limitations

As with any experiment, this study was not without its limitations. First, the statistical power was somewhat low in several response variables such as glucose and insulin due to the low population size. However, this study was novel and introductory in nature and is the first to our knowledge to begin to understand the unique metabolic effects of DF and SCFA independent to that of energy restriction which is essential to increase our understanding of obesity related lifestyle recommendations. According to these results it appears that DF and/or SCFA have the ability to alter parameters of metabolic health and deserve future investigation. Second, species differences are always a point of concern when using animals for human models. However, using the pig allowed us to more easily collect visceral tissue and provide tighter internal controls that would be difficult to accomplish in humans. Third, the daily intake of DF was beyond that of an adult's capabilities. However, the fasting concentrations of SCFA were similar to those reported in adults fed a diet high in DF. Fourth, diets were balanced with macronutrients as a percent of diet basis. Therefore, differences in macronutrient intake did occur on a g/d basis. Unfortunately, it is difficult to control for both and if we had balanced diets on a g/d basis then they would be unequal on a percent of diet basis. Fifth, gilts consumed the same feed daily. Indeed, an adult's diet would change significantly from day to day in terms of macronutrient content and it is unknown how this fact would affect the results. Sixth, no carcass

characteristics were taken. It would indeed have been beneficial to have measured total lean tissue and fat mass as well as visceral organ weight. Lastly, adipose tissue samples were not collected. Although it was not the aim of the study to determine the effects of SCFA on lipolysis and lipogenesis, the collection of adipocytes would be warranted in future studies. This would help fill the knowledge gap as to the antilipolytic or lipogenic nature of SCFA.

Conclusions

Results of this study clearly support those previously conducted in humans and pigs that DF and the subsequent production of SCFA decrease plasma concentrations of NEFA. Authors (Brighenti et al., 2006; Tarini and Wolever, 2010) have speculated that the reduction in circulating NEFA improves glucose disposal and may be a treatment/prevention method for T2DM. However, these inferences were based on acute, postprandial studies. Results from this study contradict the idea that SCFA affect fasting insulin or glucose levels, which are values used in part, to estimate diabetes risk. The present data are in agreement with other long term studies (Jenkins et al., 2002; Juntunen et al., 2003) which evaluated the effect of DF on glucose and insulin kinetics.

Results also clearly support the previous work of others who found increased levels of plasma TG due to high carbohydrate and DF. It appears that acetate is lipogenic in elevated concentrations even in periods of lower energy intake. DF is generally viewed as a "metabolically healthy" food constituent; however this study, along with others, may provide evidence that this concept may not be ubiquitous.

Diets high in DF are apt to decrease a diet's ME due to its limited energy availability and inhibitory effect on fat and protein digestion. They have also been shown to increase satiety and

subsequently reduce total caloric intake. The significant reduction in ADEI and ADG of the HF gilts in the current study certainly supports these observations.

The HF gilts had significantly less back fat when compared to ER even though there were no significant differences in ADEI. It is unclear if this is due to the numerically higher energy and lower CP intake of the ER gilts or another unknown factor.

This study provides novel data regarding the affects of DF on metabolic health independent to that of energy restriction. Results indicate that DF and the subsequent increased SCFA do have a metabolic impact outside of energy restriction and future research is warranted to better understand how they affect human health.

Table 2.1. Ingredient and nutrient composition of experimental diets: control (CON), high fiber (HF) and energy restricted (ER) (as-fed basis)

	Diet		
	CON	HF	ER
Ingredient, g/kg			
Corn	818.7	444.8	818.7
Soybean meal, 46.5%	145.4	116.8	145.4
Soybean Hulls	--	400	--
Soybean Oil	--	8.5	--
Monocalcium Phosphate, 21% P	8.5	8.5	8.5
Limestone	8.5	2.2	8.5
Salt	4.0	4.0	4.0
Vitamin Premix[1]	2.5	2.5	2.5
Mineral Premix[2]	1.5	1.5	1.5
Lysine HCl	0.9	0.7	0.9
DL-Methionine	--	0.3	--
L-Threonine	--	0.2	--
Molasses	10	10	10
Nutritional Values			
ME[3], kcal/kg	3275	2400	3275
Crude Protein,%	141	144	141
Fat, %	30	32	30
Crude Fiber, %	23	110	23
Total Dietary Fiber[4], %	185	369	185
NDF, %	74	208	74
ADF, %	32	135	32
Calcium, %	5.5	5.5	5.5
Phosphorus, %	5.0	4.5	5.0

[1]Viatmin premix provided per kilogram of diet: 6,614 IU of vitamin A, 826.7 IU of vitamin D, 26.46 IU of vitamin E, 2.65 mg of vitamin K 0.02 mg vitamin B12, 29.76 mg of niacin, 16.53 mg of pantothenic acid and 4.96 of riboflavin

[2] Trace mineral premix provided per kilogram of diet: 13.23 mg of Cu from $CuSO_4$, 0.24 mg of I from $Ca(IO_3)_2$, 132.28 mg of Fe from $FeSO_4$, 31.75 mg of Mn from $MnSO_4$, 0.24 mg of Se from $NaSeO_3$ and 132.28 mg of Zn from $ZnSO_4$

[3] Calculated using NRC (1998)

[4] Determined by AACC Approved Method 32-70 (AACC, 1991)

Table 2.2. Primers used for quantitative RT-PCR

Gene	Primer sequence (5' to 3')	Accession number
CPT1A	F-AGCCACGAGGCTGAACTGCT	NM 001129805.1
	R-ACGAACCCATTCCGCAGCGA	
CPT1B	F-ACTGTCTGGGCAAACCAAAC	NM 00100719.1
	R-CTTCTTGATGAGGCCTTTGC	
PGC-1α	F-ACCAAGAAAGGGCCCGAGCA	NM213963.1
	R-TGGCCTTCCGTTCCTCGTGT	
18S	F-CGGAACTGAGGCCATGATTA	AY265350.1
	R-TCGGAACTACGACGGTATCT	

[1] From NCBA Entrez Nucleotide Database

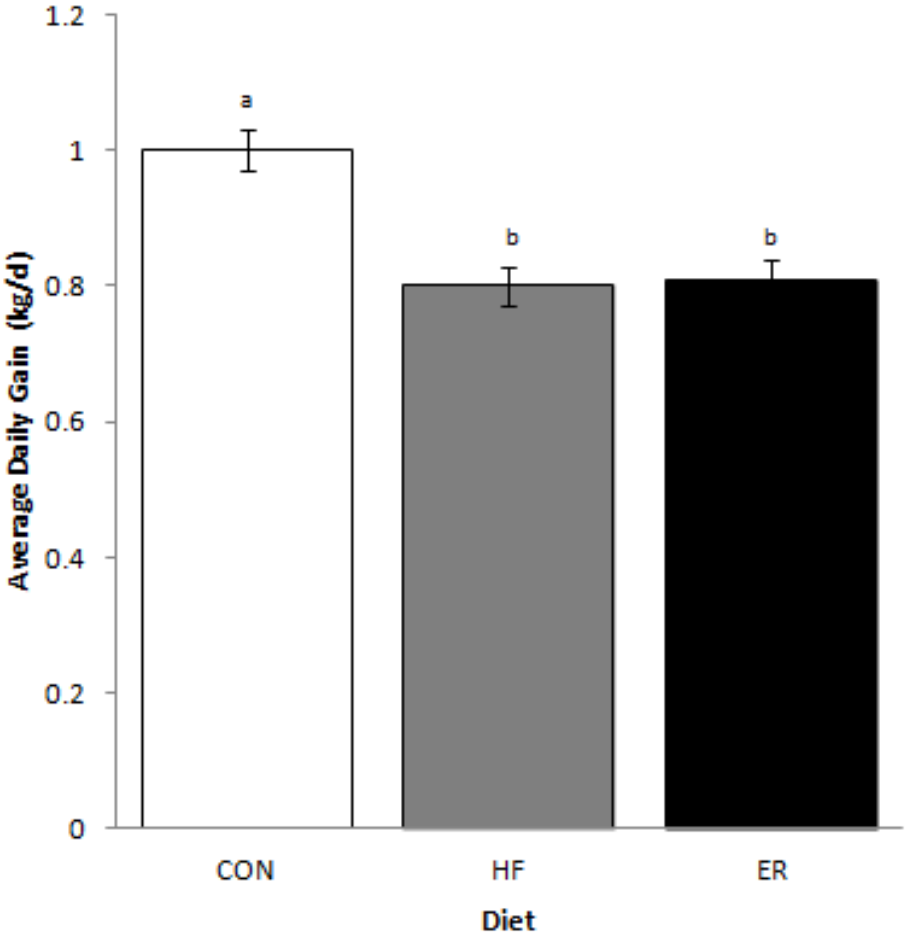
(http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide).

Table 2.3. Physical and plasma response variables in gilts before and after 42 d of a control (CON), high fiber (HF) or energy restricted (ER) diet[1]

	Diet		
Response Variable	CON	HF	ER
Body Weight (kg)			
Day 0	75.9 ± 0.8	75.1 ± 0.7	75.9 ± 0.7
Day 42	116.8 ± 1.4	108.1 ± 1.3	109.0 ± 1.3
Back Fat Thickness (cm)			
Day 0	7.0 ± 0.61	7.83 ± 0.55	6.67 ± 0.55
Day 42	13.0 ± 0.63	9.83 ± 0.57	11.17 ± 0.57
Fasting Glucose (mmol/dl)			
Day 0	4.62 ± 0.11	4.57 ± 0.10	4.49 ± 0.10
Day 42	4.37 ± 0.11	4.19 ± 0.10	4.15 ± 0.10
Fasting Insulin (ng/ml)			
Day 0	0.064 ± 0.010	0.040 ± 0.010	0.059 ± 0.010
Day 42	0.047 ± 0.008	0.041 ± 0.007	0.068 ± 0.007
Fasting TG (mg/dl)			
Day 0	18.66 ± 1.98	14.27 ± 1.81	16.95 ± 1.81
Day 42	14.10 ± 2.20	22.32 ± 2.01	14.61 ± 2.01
Fasting NEFA			
Day 0	72.99 ± 17.98	58.12 ± 16.42	39.48 ± 14.62
Day 42	70.70 ± 17.10	26.13 ± 15.61	44.32 ± 15.61
Fasting Acetate (µmol/l)			
Day 0	152.40 ± 9.04	143.08 ± 8.25	137.54 ± 8.25
Day 42	154.68 ± 12.28	259.03 ± 11.21	142.96 ± 11.21
Fasting Propionate (µmol/l)			
Day 0	7.52 ± 0.28	7.76 ± 0.25	7.66 ± 0.25
Day 42	7.20 ± 0.31	9.33 ± 0.28	6.11 ± 0.28
Fasting Butyrate (µmol/l)			
Day 0	8.02 ± 0.84	7.62 ± 0.77	8.47 ± 0.77
Day 42	6.13 ± 0.84	10.90 ± 0.76	7.07 ± 0.76

[1] Values are means ± SEM

Figure 2.1. Average daily gain (kg/d) of gilts after 42 d of a control (CON), high fiber (HF) or energy restricted diet [1,2,3]

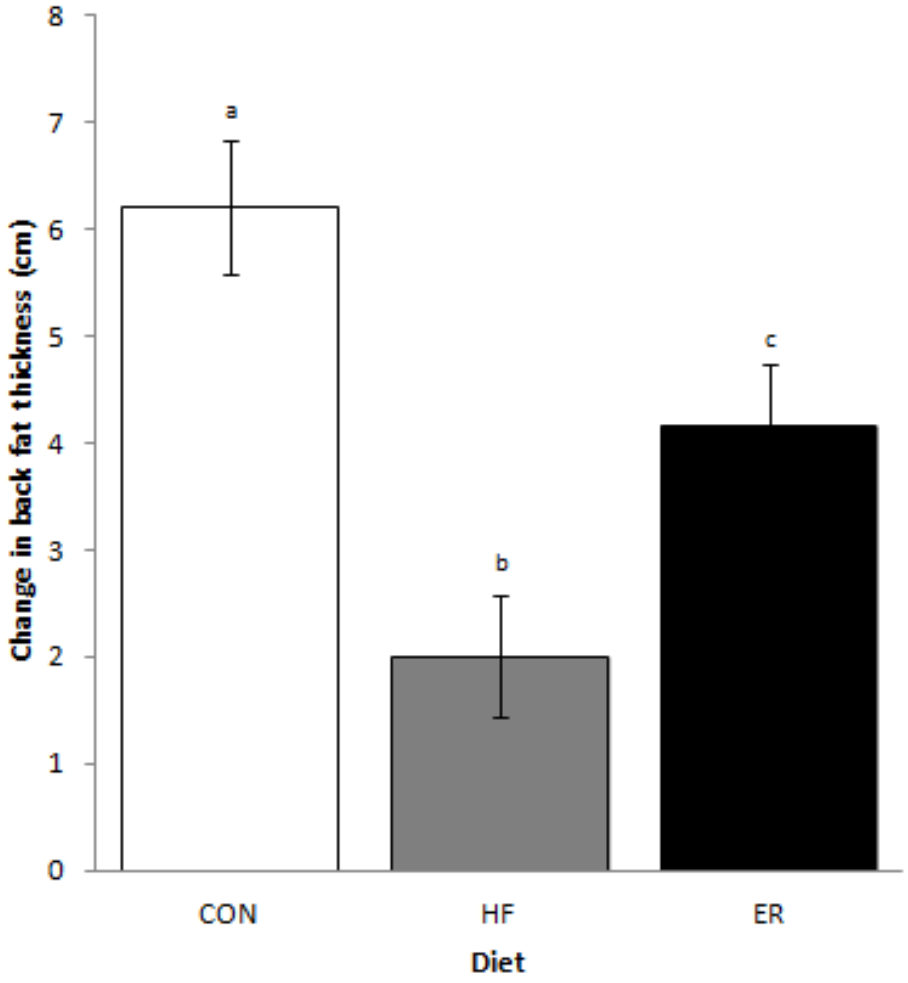


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly ($P < 0.05$) in their difference between the means

Figure 2.2. Change in back fat thickness [2] (cm) after 42 d of a control (CON), high fiber (HF) or energy restricted (ER) diet [1,3,4]



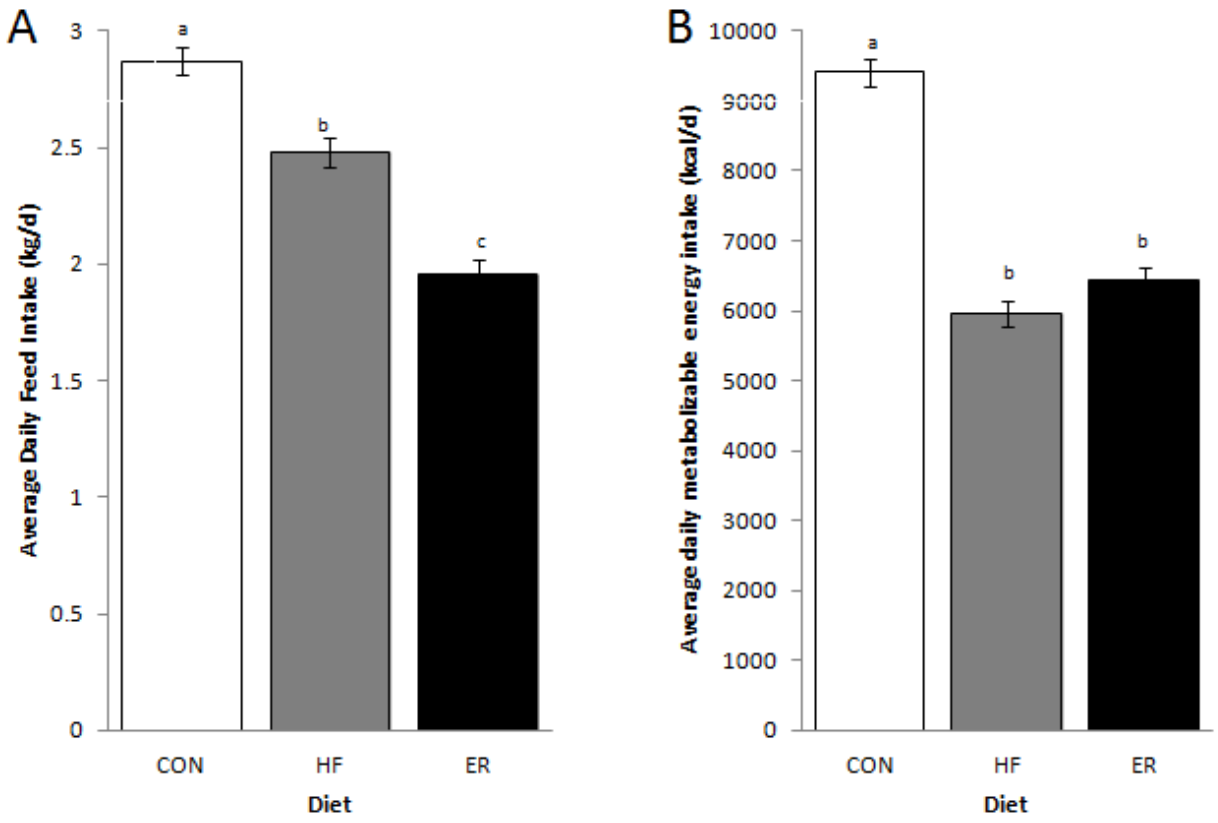
[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Back fat thickness was measured at the 14th rib via ultrasound (Aloka 500, 5 mHz linear array transducer, Wallingford, CT).

[3] Value are means ± SEM; n = 17.

[4] Values lacking a common superscript letter differ significantly ($P < 0.05$) in their difference between the means.

Figure 2.3. Average daily feed intake (A) and average daily metabolizable energy intake (B) in gilts consuming a control (CON), high fiber (HF) or energy restricted (ER) diet [1,2,3]

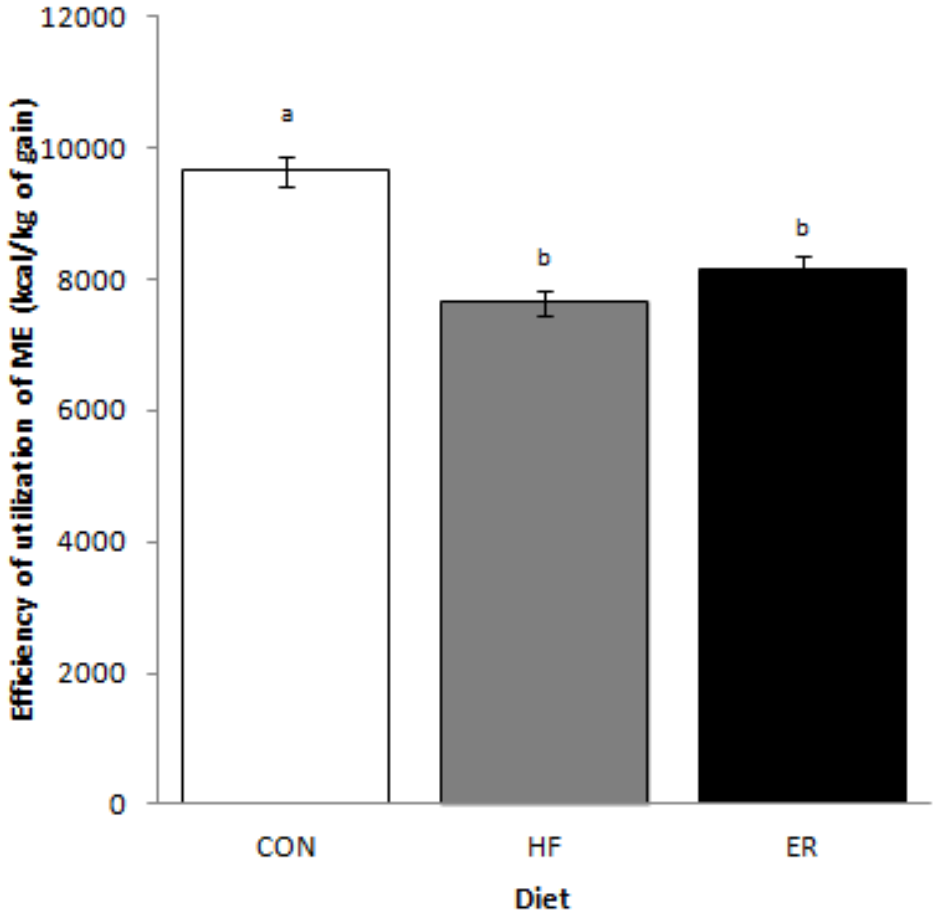


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly ($P < 0.05$) in their difference between the means

Figure 2.4. Energy efficiency of gilts consuming a control (CON), high fiber (HF) or energy restricted (ER) diet[1,2,3]

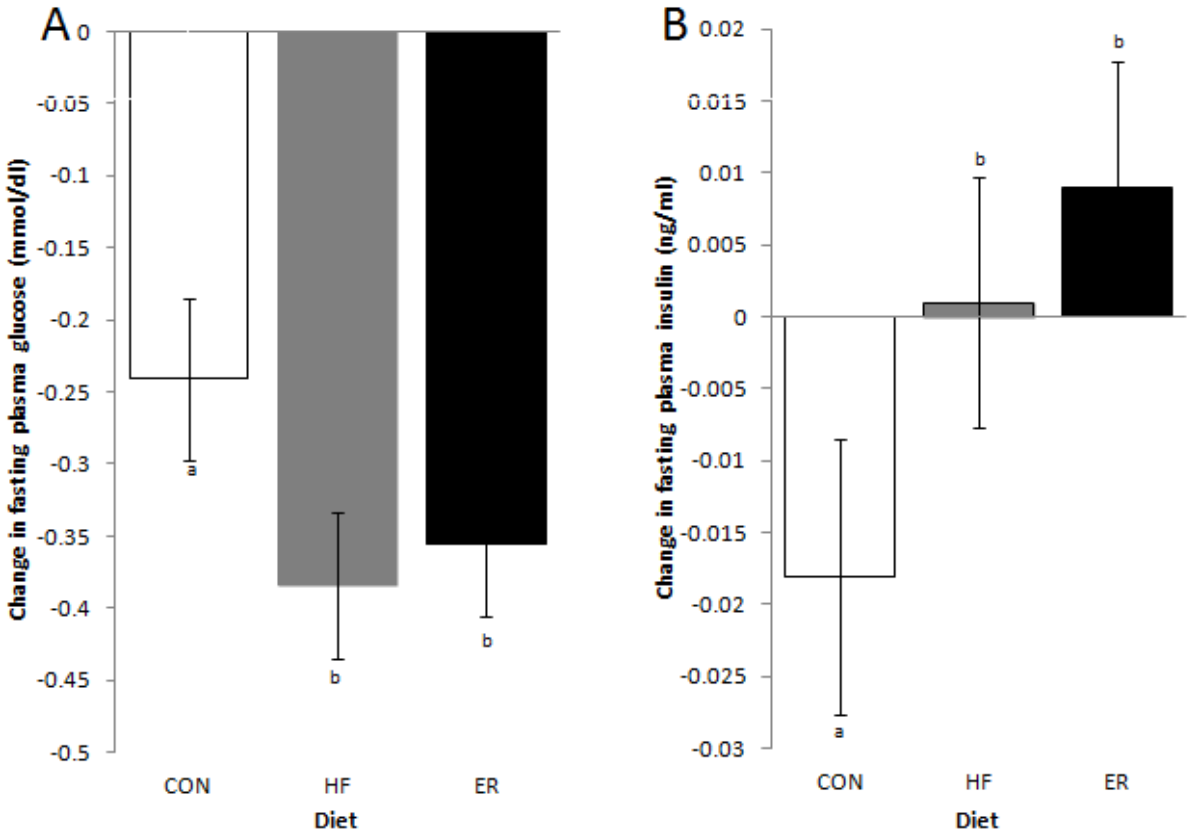


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly (P <0.05) in their difference between the means

Figure 2.5. Change in fasting plasma glucose (A) and insulin (B) concentrations in gilts after 42 d of a control (CON), high fiber (HF) or energy restricted (ER) diet[1,2,3]

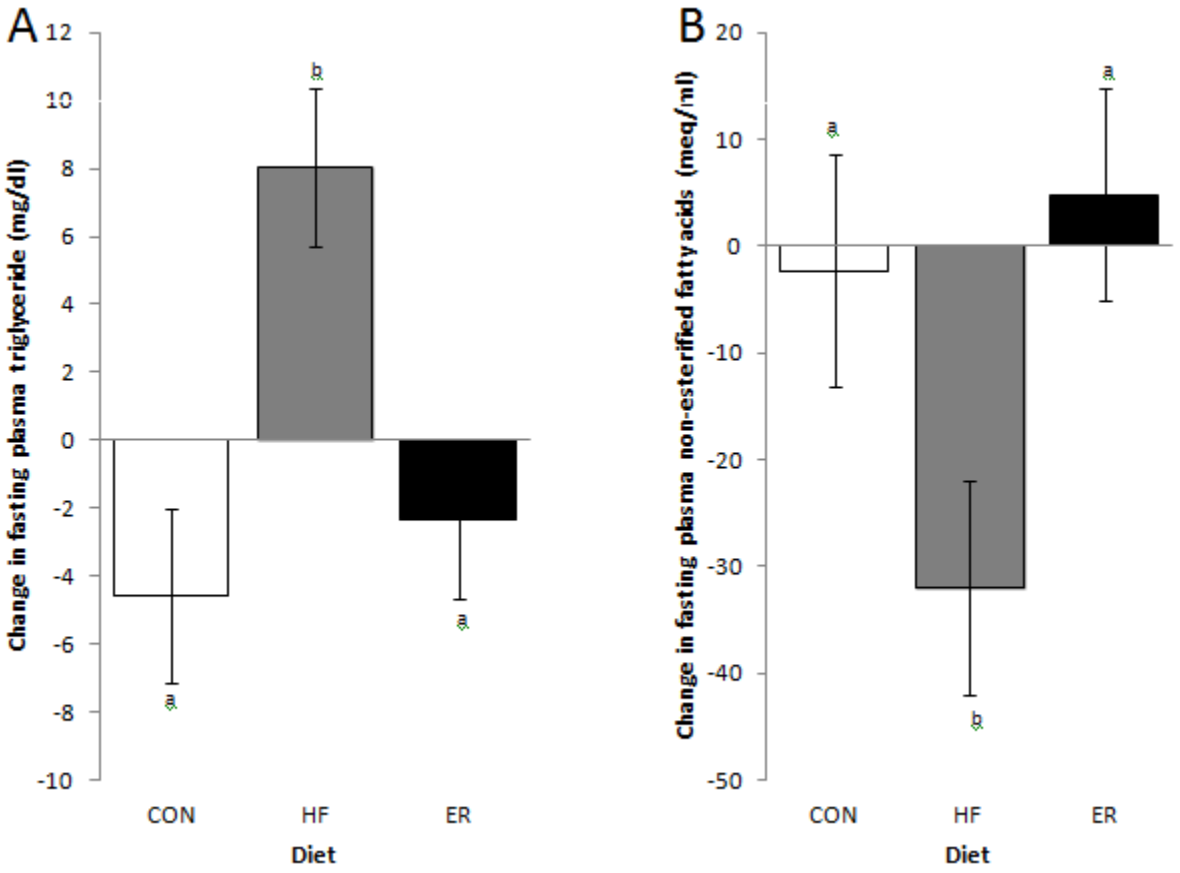


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly ($P < 0.05$) in their difference between the means

Figure 2.6. Change in fasting plasma triglyceride (A) and non-esterifed fatty acids (B) concentrations in gilts after 42 d of a control (CON), high fiber (HF), or energy restricted (ER) diet[1,2,3]

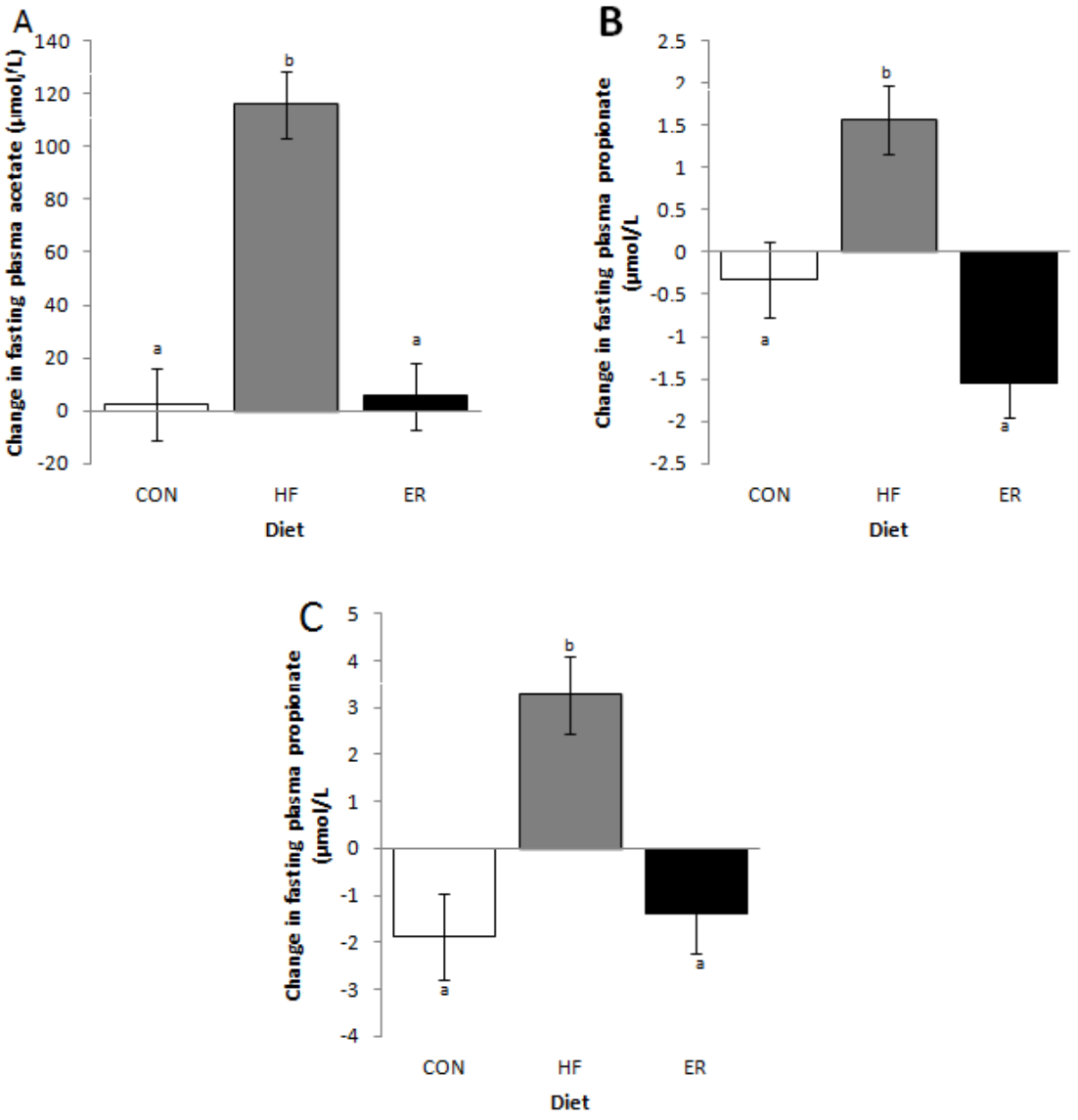


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly (P <0.05) in their difference between the means

Figure 2.7. Change in fasting acetate (A), propionate (B) and butyrate (C) concentrations (mmol/l) in gilts after 42 d of a control (CON), high fiber (HF), or energy restricted (ER) diet[1,2,3]

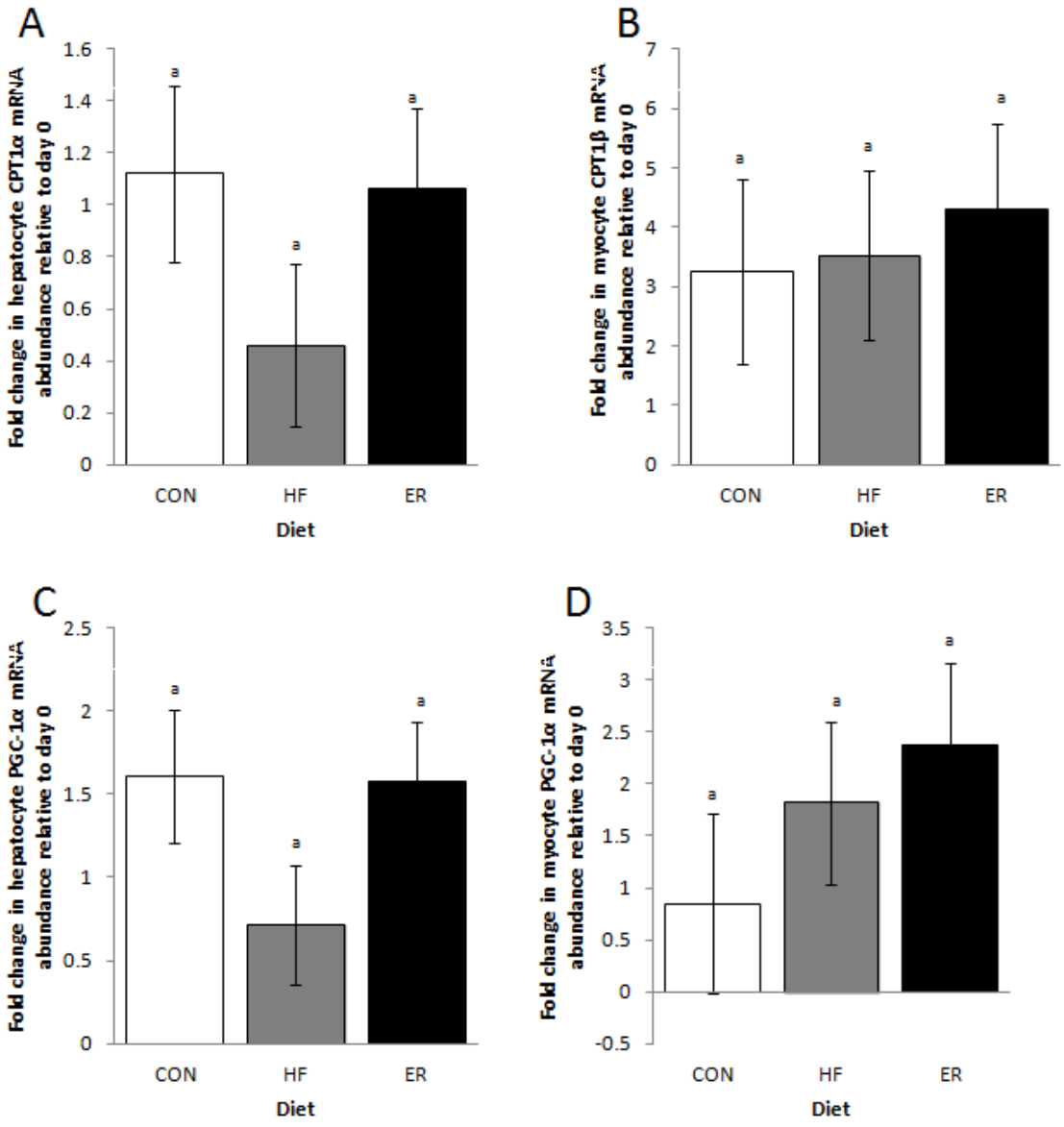


[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly (P <0.05) in their difference between the means

Figure 2.8. Fold change in hepatocyte CPT1α (A), mycocyte CPT1β (B), hepatocyte PGC-1α (C), and myocyte PGC-1α (D) mRNA abundance relative to day 0 in gilts after 42 d of a control (CON), high fiber (HF), or energy restricted (ER) diet[1,2,3]



[1] Corresponding metabolizable energy (kcal/kg) and TDF (g/kg, Total Dietary Fiber) were 2860 & 23.0, 2508 & 11.0, and 2860 & 23.0, respectively.

[2] Value are means ± SEM; n = 17.

[3] Values lacking a common superscript letter differ significantly ($P < 0.05$) in their difference between the means

Chapter 3 - Personal Reflection

I will preface my personal reflection chapter with the statement that my research entails only the effects of DF on the metabolic health parameters of adiposity. I have not and will not make mention as to the colonic and anti-carcinogenic benefits of DF. Certainly, these are entirely different implications of DF. Although I will admit that evidence exists which links microbiota of the large intestine to metabolic health.

Energy balance

Upon writing my review paper of the metabolic benefits of DF (see Chapter 1) I took on the attitude that DF was indeed a "healthy" food constituent. After all, epidemiological data overwhelming supports this theory. If we agree that metabolic health is measured by the biomarkers of adiposity, lipidemia and glucose/insulin kinetics then DF shows a strong indirect association with the factors of metabolic syndrome. However, when one begins to investigate the affects of DF in a clinical setting their implications become less clear. This is when my position on DF began to change.

I have no doubt that DF could decrease a person's relative risk for metabolic syndrome. However, this statement comes with certain requirements. First, the incorporation of DF in a diet must decrease a person's daily energy intake. This can be accomplished by increasing satiety or decreasing the energy density of the diet. Second, this decrease in energy intake must be sufficient enough to create a eucaloric or hypocaloric state. If a person consumes the USDA suggested 14 g/1000 kcal during a positive energy balance then it is likely that no metabolic benefits would be obtained. There is no evidence to suggest that DF could treat or prevent

metabolic syndrome beyond that of energy restriction. Energy balance is key regardless of how it is accomplished.

DF is simply an avenue in which to maintain a euclaoric or hypocaloric state. Sure, DF can be a healthy option; however it is not "required" to be healthy. This is demonstrated in low carbohydrate diets. Many studies agree that a low carbohydrate lifestyle can improve metabolic health factors beyond that of a low fat lifestyle.

It is unrealistic to expect every American to consume 14g/1000 kcal of DF. Not only does DF decrease the palatability of a food but in addition it creates gastric discomfort such as gas and bloating.

Short chain fatty acids

Much more is known about SCFA metabolism in animals, specifically ruminants. Due to the limited amount of invasive procedures that can be conducted in humans far less is known about this 2, 3 and 4 carbon fatty acids. Acetate can be rapidly oxidized for energy via the TCA cycle or undergo lipogenesis during periods of a positive energy balance. Propionate is another easily oxidized energy source and can also undergo gluconeogenesis. Butyrate is generally thought to be primarily thought to be the preferred energy source of coloncytes. However, in the case of my study, high levels of butyrate can bypass this fate and enter peripheral circulation.

Several researchers theorize that SCFA can treat or prevent type 2 diabetes. Their postulate is based on acute, postprandial, studies that demonstrate SCFA's ability to inhibit plasma NEFA. It is my opinion that this is a temporary fix and in the long run will have minimal affects on glucose and insulin kinetics. In order to truly treat or prevent this disorder without pharmacological interventions, a person must control their energy state. SCFA would likely contribute to the body's ATP supply and not create a negative energy or energy balanced state.

Simply lowering postprandial NEFA concentrations as suggested by others would likely have minimal long term effects on type 2 diabetes.

Carbohydrate chemistry and rheology

I dramatically increased my knowledge of carbohydrate chemistry and rheology over the course of my doctoral work. This proved to be invaluable during my research as the structure and functionality of a carbohydrate can greatly affect its digestibility whereby altering its metabolic impact. This is a fact that is underappreciated and misunderstood by many nutritionists.

Altering a carbohydrate which will limit is digestion and absorption in the small intestine has a direct impact on energy balance, glucose and insulin kinetics, lipidemia and gut health. Unfortunately, when small intestinal digestibility is decreased it commonly results in gastric discomforts such as gas, bloating, abdominal cramps, etc. Nevertheless, future research is warranted regarding carbohydrate chemistry and metabolic health. The production of a carbohydrate that is neither digested absorbed in the small intestine, produces no gastric discomfort, and palatable would be a remarkable invention.

References – Chapter 1

1. Tucker, L.A.; Thomas, K.S. Increasing total fiber intake reduces risk of weight and fat gains in women. *J. Nutr.* **2009**, *139*, 576-581.
2. Meyer, K.A.; Kushi, L.H.; Jacobs, D.R., Jr.; Slavin, J.; Sellers, T.A.; Folsom, A.R. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *Am. J. Clin. Nutr.* **2000**, *71*, 921-930.
3. Park, Y.; Brinton, L.A.; Subar, A.F.; Hollenbeck, A.; Schatzkin, A. Dietary fiber intake and risk of breast cancer in postmenopausal women: the National Institutes of Health-AARP Diet and Health Study. *Am. J. Clin. Nutr.* **2009**, *90*, 664-671.
4. Streppel, M.T.; Ocke, M.C.; Boshuizen, H.C.; Kok, F.J.; Kromhout, D. Dietary fiber intake in relation to coronary heart disease and all-cause mortality over 40 y: the Zutphen Study. *Am. J. Clin. Nutr.* **2008**, *88*, 1119-1125.
5. FDA, Health claims: fiber-contaning grain products, fruits and vegetables and cancer. In Code of Federal Regulations, Food and Drug Administration: 2008; Vol. 2.
6. Nomura, A.M.; Hankin, J.H.; Henderson, B.E.; Wilkens, L.R.; Murphy, S.P.; Pike, M.C.; Le Marchand, L.; Stram, D.O.; Monroe, K.R.; Kolonel, L.N. Dietary fiber and colorectal cancer risk: the multiethnic cohort study. *C. C. C.* **2007**, *18*, 753-764.
7. Schatzkin, A.; Park, Y.; Leitzmann, M.F.; Hollenbeck, A.R.; Cross, A.J. Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. *Gastroenterology* **2008**, *135*, 1163-1167.
8. Young, G.P.; Hu, Y.; Le Leu, R.K.; Nyskohus, L. Dietary fibre and colorectal cancer: A model for environment - gene interactions. *Mol. Nutr. Food Res.* **2005**, *49*, 571-584.
9. Adlercreutz, H.; Hamalainen, E.; Gorbach, S.L.; Goldin, B.R.; Woods, M.N.; Brunson, L.S.; Dwyer, J.T. Association of Diet and Sex-Hormones in Relation to Breast-Cancer. *Eur. J. Cancer Clin. On.* **1987**, *23*, 1725-1726.
10. FDA, Health claims: fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease. In Code of Federal Regulations, Food and Drug Administration: 2008; Vol. 2.
11. Pereira, M.A.; O'Reilly, E.; Augustsson, K.; Fraser, G.E.; Goldbourt, U.; Heitmann, B.L.; Hallmans, G.; Knekt, P.; Liu, S.M.; Pietinen, P.; Spiegelman, D.; Stevens, J.; Virtamo, J.; Willett, W.C.; Ascherio, A. Dietary fiber and risk of coronary heart disease - A pooled analysis of cohort studies. *Arch. Intern. Med.* **2004**, *164*, 370-376.
12. Streppel, M.T.; Ocke, M.C.; Boshuizen, H.C.; Kok, F.J.; Kromhout, D. Dietary fiber intake in relation to coronary heart disease and all-cause mortality over 40 y: the Zutphen Study. *Am. J. Clin. Nutr.* **2008**, *88*, 1119-1125.
13. Story, J.A.; Furumoto, E.J.; Buhman, K.K. Dietary fiber and bile acid metabolism - an update. *Adv. Exp. Med. Biol.* **1997**, *427*, 259-266.
14. Amaral, L.; Morgan, D.; Stephen, A.M.; Whiting, S. Effect of Propionate on Lipid-Metabolism in Healthy-Human Subjects. *Faseb. J.* **1992**, *6*, A1655-A1655.
15. Esposito, K.; Nappo, F.; Giugliano, F.; Di Palo, C.; Ciotola, M.; Barbieri, M.; Paolisso, G.; Giugliano, D. Meal modulation of circulating interleukin 18 and adiponectin concentrations in healthy subjects and in patients with type 2 diabetes mellitus. *Am. J. Clin. Nutr.* **2003**, *78*, 1135-1140.

16. Ma, Y.S.; Griffith, J.A.; Chasan-Taber, L.; Olendzki, B.C.; Jackson, E.; Stanek, E.J.; Li, W.J.; Pagoto, S.L.; Hafner, A.R.; Ockene, I.S. Association between dietary fiber and serum C-reactive protein. *Am. J. Clin. Nutr.* **2006**, *83*, 760-766.
17. Ogden, C.L.; Carroll, M.D.; Curtin, L.R.; McDowell, M.A.; Tabak, C.J.; Flegal, K.M. Prevalence of overweight and obesity in the United States, 1999-2004. *J. Am. Med. Assoc.* **2006**, *295*, 1549-1555.
18. Mokdad, A.H.; Ford, E.S.; Bowman, B.A.; Dietz, W.H.; Vinicor, F.; Bales, V.S.; Marks, J.S. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *J. Am. Med. Assoc.* **2003**, *289*, 76-79.
19. Weickert, M.O.; Pfeiffer, A.F.H. Metabolic effects of dietary and prevention of diabetes. *J. Nutr.* **2008**, *138*, 439-442.
20. Chemists, A.A.o.C. AACC adopts oat bran definition. Available online: (October 12),
21. DeVries, J.W. On defining dietary fibre. *P. Nutr. Soc.* **2003**, *62*, 37-43.
22. FAO/WHO Codex Alimentarius Commission. Available online: (October 12),
23. Jones, J. Update on defining dietary fiber. *Cereal Food World* **2000**, *45*, 219-220.
24. Sizer, F.; Whitney, E., *Nutrition:Concepts and Controversies*; 11 ed.; Thomson Wadsworth: Belmont, Ca, 2008.
25. Wong, J.M.; Jenkins, D.J. Carbohydrate digestibility and metabolic effects. *J. Nutr.* **2007**, *137*, 2539S-2546S.
26. Liu, R.H. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78*, 517s-520s.
27. Weickert, M.O.; Pfeiffer, A.F. Metabolic effects of dietary fiber consumption and prevention of diabetes. *J. Nutr.* **2008**, *138*, 439-442.
28. Liu, S.; Stampfer, M.J.; Hu, F.B.; Giovannucci, E.; Rimm, E.; Manson, J.E.; Hennekens, C.H.; Willett, W.C. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am. J. Clin. Nutr.* **1999**, *70*, 412-419.
29. Ferguson, L.R.; Chavan, R.R.; Harris, P.J. Changing concepts of dietary fiber: implications for carcinogenesis. *Nutr. Cancer* **2001**, *39*, 155-169.
30. Terry, P.; Giovannucci, E.; Michels, K.B.; Bergkvist, L.; Hansen, H.; Holmberg, L.; Wolk, A. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *J. Natl. Cancer Inst.* **2001**, *93*, 525-533.
31. Li, Z.; Bowerman, S.; Heber, D. Health ramifications of the obesity epidemic. *Surg. Clin. North Am.* **2005**, *85*, 681-701, v.
32. Koh-Banerjee, P.; Franz, M.V.; Sampson, L.; Liu, S.M.; Jacobs, D.R.; Spiegelman, D.; Willett, W.; Rimm, E. Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. *Am. J. Clin. Nutr.* **2004**, *80*, 1237-1245.
33. Keenan, H.A.; Doria, A.; Aiello, L.P.; King, G.L. Positivity of C-peptide, GADA and IA2 antibodies in type 1 diabetic patients with extreme duration. *Diabetes* **2006**, *55*, A65-A65.
34. Baer, D.J.; Rumpler, W.V.; Miles, C.W.; Fahey, G.C. Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. *J. Nutr.* **1997**, *127*, 579-586.
35. Isken, F.; Klaus, S.; Osterhoff, M.; Pfeiffer, A.F.H.; Weickert, M.O. Effects of long-term soluble *vs.* insoluble dietary fiber intake on high-fat diet-induced obesity in C57BL/6J mice. *J. Nutr. Biochem.* **2010**, *21*, 278-284.
36. Davidson, M.H.; McDonald, A. Fiber: Forms and functions. *Nutr. Res.* **1998**, *18*, 617-624.
37. Schneeman, B.O. Dietary fiber and gastrointestinal function. *Nutr. Res.* **1998**, *18*, 625-632.

38. Renteria-Flores, J.A.; Johnston, L.J.; Shurson, G.C.; Gallaher, D.D. Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows. *J. Anim. Sci.* **2008**, *86*, 2568-2575.
39. Anderson, J.W. Physiological and Metabolic Effects of Dietary Fiber. *Fed. Proc.* **1985**, *44*, 2902-2906.
40. Keenan, M.J.; Zhou, J.; McCutcheon, K.L.; Raggio, A.M.; Bateman, H.G.; Todd, E.; Jones, C.K.; Tulley, R.T.; Melton, S.; Martin, R.J.; Hegsted, M. Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity (Silver Spring)* **2006**, *14*, 1523-1534.
41. Du, H.D.; van der A, D.L.; Boshuizen, H.C.; Forouhi, N.G.; Wareham, N.J.; Halkjaer, J.; Tjonneland, A.; Overvad, K.; Jakobsen, M.U.; Boeing, H.; Buijsse, B.; Masala, G.; Palli, D.; Sorensen, T.I.A.; Saris, W.H.M.; Feskens, E.J.M. Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am. J. Clin. Nutr.* **2010**, *91*, 329-336.
42. Meyer, K.A.; Kushi, L.H.; Jacobs, D.R.; Slavin, J.; Sellers, T.A.; Folsom, A.R. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *Am. J. Clin. Nutr.* **2000**, *71*, 921-930.
43. Hu, F.B.; Manson, J.E.; Stampfer, M.J.; Colditz, G.; Liu, S.; Solomon, C.G.; Willett, W.C. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New Engl. J. Med.* **2001**, *345*, 790-797.
44. Schulze, M.B.; Liu, S.; Rimm, E.B.; Manson, J.E.; Willett, W.C.; Hu, F.B. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *Am. J. Clin. Nutr.* **2004**, *80*, 348-356.
45. Jenkins, D.J.; Wolever, T.M.; Leeds, A.R.; Gassull, M.A.; Haisman, P.; Dilawari, J.; Goff, D.V.; Metz, G.L.; Alberti, K.G. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br. Med. J.* **1978**, *1*, 1392-1394.
46. Montonen, J.; Knekt, P.; Jarvinen, R.; Aromaa, A.; Reunanen, A. Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am. J. Clin. Nutr* **2003**, *77*, 622-629.
47. Salmeron, J.; Ascherio, A.; Rimm, E.B.; Colditz, G.A.; Spiegelman, D.; Jenkins, D.J.; Stampfer, M.J.; Wing, A.L.; Willett, W.C. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* **1997**, *20*, 545-550.
48. Weickert, M.O.; Mohlig, M.; Koebnick, C.; Holst, J.J.; Namsolleck, P.; Ristow, M.; Osterhoff, M.; Rochlitz, H.; Rudovich, N.; Spranger, J.; Pfeiffer, A.F. Impact of cereal fibre on glucose-regulating factors. *Diabetologia* **2005**, *48*, 2343-2353.
49. Samra, R.; Anderson, G.H. Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men. *Am. J. Clin. Nutr.* **2007**, *86*, 972-979.
50. Brighenti, F.; Castellani, G.; Benini, L.; Casiraghi, M.C.; Leopardi, E.; Crovetti, R.; Testolin, G. Effect of Neutralized and Native Vinegar on Blood-Glucose and Acetate Responses to a Mixed Meal in Healthy-Subjects. *Eur. J. Clin. Nutr.* **1995**, *49*, 242-247.
51. Ostman, E.M.; Liljeberg Elmstahl, H.G.; Bjorck, I.M. Barley bread containing lactic acid improves glucose tolerance at a subsequent meal in healthy men and women. *J. Nutr.* **2002**, *132*, 1173-1175.
52. Ferrannini, E.; Barrett, E.J.; Bevilacqua, S.; DeFronzo, R.A. Effect of fatty acids on glucose production and utilization in man. *J. Clin. Invest.* **1983**, *72*, 1737-1747.

53. Crouse, J.R.; Gerson, C.D.; Decarli, L.; Lieber, C.S. Role of Acetate in Reduction of Plasma Free Fatty Acids Produced by Ethanol in Man. *J. Lipid. Res.* **1968**, *9*, 509-&.
54. Kelley, D.E.; Mandarino, L.J. Fuel selection in human skeletal muscle in insulin resistance - A reexamination. *Diabetes* **2000**, *49*, 677-683.
55. Slavin, J.L.; Martini, M.C.; Jacobs, D.R., Jr.; Marquart, L. Plausible mechanisms for the protectiveness of whole grains. *Am. J. Clin. Nutr* **1999**, *70*, 459S-463S.
56. Paolisso, G.; Barbagallo, M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. *Am. J. Hypertens.* **1997**, *10*, 346-355.
57. Jenkins, D.J.A.; Kendall, C.W.C.; Augustin, L.S.A.; Martini, M.C.; Axelsen, M.; Faulkner, D.; Vidgen, E.; Parker, T.; Lau, H.; Connelly, P.W.; Teitel, J.; Singer, W.; Vandenbroucke, A.C.; Leiter, L.A.; Josse, R.G. Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. *Diabetes Care* **2002**, *25*, 1522-1528.
58. Ring, S.G.; Selvendran, R.R. Isolation and Analysis of Cell-Wall Material from Beeswing Wheat Bran (Triticum-Aestivum). *Phytochemistry* **1980**, *19*, 1723-1730.
59. Lu, Z.X.; Walker, K.Z.; Muir, J.G.; Mascara, T.; O'Dea, K. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *Am. J. Clin. Nutr.* **2000**, *71*, 1123-1128.
60. Lu, Z.X.; Walker, K.Z.; Muir, J.G.; O'Dea, K. Arabinoxylan fibre improves metabolic control in people with Type II diabetes. *Eur. J. Clin. Nutr.* **2004**, *58*, 621-628.
61. Gibson, G.R.; Beatty, E.R.; Wang, X.; Cummings, J.H. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **1995**, *108*, 975-982.
62. Rafter, J.; Bennett, M.; Caderni, G.; Clune, Y.; Hughes, R.; Karlsson, P.C.; Klinder, A.; O'Riordan, M.; O'Sullivan, G.C.; Pool-Zobel, B.; Rechkemmer, G.; Roller, M.; Rowland, I.; Salvadori, M.; Thijs, H.; Van Loo, J.; Watzl, B.; Collins, J.K. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *Am. J. Clin. Nutr.* **2007**, *85*, 488-496.
63. Griffin, I.J.; Hicks, P.M.D.; Heaney, R.P.; Abrams, S.A. Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. *Nutr. Res.* **2003**, *23*, 901-909.
64. Abrams, S.A.; Griffin, I.J.; Hawthorne, K.M.; Liang, L.; Gunn, S.K.; Darlington, G.; Ellis, K.J. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am. J. Clin. Nutr.* **2005**, *82*, 471-476.
65. Cani, P.D.; Joly, E.; Horsmans, Y.; Delzenne, N.M. Oligofructose promotes satiety in healthy human: a pilot study. *Eur. J. Clin. Nutr.* **2006**, *60*, 567-572.
66. Wood, P.J.; Weisz, J.; Fedec, P. Potential for Beta-Glucan Enrichment in Brans Derived from Oat (Avena-Sativa L) Cultivars of Different (1-]3),(1-]4)-Beta-D-Glucan Concentrations. *Cereal Chem.* **1991**, *68*, 48-51.
67. Doublier, J.L.; Wood, P.J. Rheological Properties of Aqueous-Solutions of (1-]3)(1-]4)-Beta-D-Glucan from Oats (Avena-Sativa L). *Cereal Chem.* **1995**, *72*, 335-340.
68. Theuwissen, E.; Mensink, R.P. Simultaneous intake of beta-glucan and plant stanol esters affects lipid metabolism in slightly hypercholesterolemic subjects. *J. Nutr.* **2007**, *137*, 583-588.

69. Naumann, E.; van Rees, A.B.; Onning, G.; Oste, R.; Wydra, M.; Mensink, R.P. beta-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations. *Am. J. Clin. Nutr.* **2006**, *83*, 601-605.
70. Biorklund, M.; van Rees, A.; Mensink, R.P.; Onning, G. Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with beta-glucans from oats or barley: a randomised dose-controlled trial. *Eur. J. Clin. Nutr.* **2005**, *59*, 1272-1281.
71. Tappy, L. Regulation of hepatic glucose production in healthy subjects and patients with non-insulin-dependent diabetes mellitus. *Diabete Metab.* **1995**, *21*, 233-240.
72. Lia, A.; Hallmans, G.; Sandberg, A.S.; Sundberg, B.; Aman, P.; Andersson, H. Oat beta-glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. *Am. J. Clin. Nutr.* **1995**, *62*, 1245-1251.
73. Nazare, J.A.; Normand, S.; Triantafyllou, A.O.; de la Perriere, A.B.; Desage, M.; Laville, M. Modulation of the postprandial phase by beta-glucan in overweight subjects: Effects on glucose and insulin kinetics. *Mol. Nutr. Food Res.* **2009**, *53*, 361-369.
74. Kim, H.J.; White, P.J. In Vitro Bile-Acid Binding and Fermentation of High, Medium, and Low Molecular Weight beta-Glucan. *J. Agr. Food Chem.* **2010**, *58*, 628-634.
75. Kim, H.J.; White, P.J. In Vitro Fermentation of Oat Flours from Typical and High beta-Glucan Oat Lines. *J. Agr. Food Chem.* **2009**, *57*, 7529-7536.
76. Ide, T.; Okamatsu, H.; Sugano, M. Regulation by Dietary Fats of 3-Hydroxy-3-Methylglutaryl-Coenzyme-a Reductase in Rat-Liver. *J. Nutr.* **1978**, *108*, 601-612.
77. Keogh, G.F.; Cooper, G.J.S.; Mulvey, T.B.; McArdle, B.H.; Coles, G.D.; Monro, J.A.; Poppitt, S.D. Randomized controlled crossover study of the effect of a highly beta-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *Am. J. Clin. Nutr.* **2003**, *78*, 711-718.
78. Cugnet-Anceau, C.; Nazare, J.A.; Biorklund, M.; Le Coquil, E.; Sassolas, A.; Sothier, M.; Holm, J.; Landin-Olsson, M.; Onning, G.; Laville, M.; Moulin, P. A controlled study of consumption of beta-glucan-enriched soups for 2 months by type 2 diabetic free-living subjects. *Br. J. Nutr.* **2010**, *103*, 422-428.
79. Suortti, T.; Johansson, L.; Autio, K., Effect of heating and freezing on molecular weight of oat β-glucan. In *AACC Annual Meeting*, 2000.
80. Kerckhoffs, D.A.J.M.; Hornstra, G.; Mensink, R.P. Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. *Am. J. Clin. Nutr.* **2003**, *78*, 221-227.
81. Yao, N.; Jannink, J.L.; White, P.J. Molecular weight distribution of (1 -> 3)(1 -> 4)-beta-Glucan affects pasting properties of flour from oat lines with high and typical amounts of beta-glucan. *Cereal Chem.* **2007**, *84*, 471-479.
82. Gaidosova, A.; Petruldkova, Z.; HavrlentoVd, M.; Ervend, V.; HozoVd, B.; Turdik, E.; Kogan, G. The content of water-soluble and water-insoluble beta-D-glucans in selected oats and barley varieties. *Carbohyd. Polym.* **2007**, *70*, 46-52.
83. Torronen, R.; Kansanen, L.; Uusitupa, M.; Hanninen, O.; Myllymaki, O.; Harkonen, H.; Malkki, Y. Effects of an oat bran concentrate on serum lipids in free-living men with mild to moderate hypercholesterolaemia. *Eu.r J. Clin. Nutr.* **1992**, *46*, 621-627.
84. Cugnet-Anceau, C.; Nazare, J.A.; Biorklund, M.; Le Coquil, E.; Sassolas, A.; Sothier, M.; Holm, J.; Landin-Olsson, M.; Onning, G.; Laville, M.; Moulin, P. A controlled study of

consumption of beta-glucan-enriched soups for 2 months by type 2 diabetic free-living subjects. *Brit. J. Nutr.* **2010**, *103*, 422-428.
85. Braaten, J.T.; Wood, P.J.; Scott, F.W.; Wolynetz, M.S.; Lowe, M.K.; Bradley-White, P.; Collins, M.W. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* **1994**, *48*, 465-474.
86. Lawaetz, O.; Blackburn, A.M.; Bloom, S.R.; Aritas, Y.; Ralphs, D.N.L. Effect of Pectin on Gastric-Emptying and Gut Hormone-Release in the Dumping Syndrome. *Scand. J. Gastroentero.* **1983**, *18*, 327-336.
87. Brown, L.; Rosner, B.; Willett, W.W.; Sacks, F.M. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am. J. Clin. Nutr.* **1999**, *69*, 30-42.
88. Jenkins, D.J.A.; Leeds, A.R.; Gassull, M.A.; Cochet, B.; Alberti, K.G.M.M. Decrease in Postprandial Insulin and Glucose Concentrations by Guar and Pectin. *Ann. Intern. Med.* **1977**, *86*, 20-23.
89. Rabbani, G.H.; Teka, T.; Zaman, B.; Majid, N.; Khatun, M.; Fuchs, G.J. Clinical studies in persistent diarrhea: Dietary management with green banana or pectin in Bangladeshi children. *Gastroenterology* **2001**, *121*, 554-560.
90. Triplehorn, C.; Millard, P.S. A rice-based diet with green banana or pectin reduced diarrhea in infants better than a rice-alone diet. *ACP. J. Club* **2002**, *136*, 67.
91. Olano-Martin, E.; Gibson, G.R.; Rastell, R.A. Comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. *J. Appl. Microbiol.* **2002**, *93*, 505-511.
92. Veldman, F.J.; Nair, C.H.; Vorster, H.H.; Vermaak, W.J.H.; Jerling, J.C.; Oosthuizen, W.; Venter, C.S. Possible mechanisms through which dietary pectin influences fibrin network architecture in hypercholesterolaemic subjects. *Thromb. Res.* **1999**, *93*, 253-264.
93. Nangia-Makker, P.; Hogan, V.; Honjo, Y.; Baccarini, S.; Tait, L.; Bresalier, R.; Raz, A. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *J. Natl. Cancer. I* **2002**, *94*, 1854-1862.
94. AACC AACC adopts oat bran definition. Available online: (October 12),
95. Ulmius, M.; Johansson, A.; Onning, G. The influence of dietary fibre source and gender on the postprandial glucose and lipid response in healthy subjects. *Eur. J. Nutr.* **2009**, *48*, 395-402.
96. Qureshi, A.A.; Sami, S.A.; Khan, F.A. Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus Types I and II. *J. Nutr. Biochem.* **2002**, *13*, 175-187.
97. Zhang, J.X.; Lundin, E.; Hallmans, G.; Adlercreutz, H.; Andersson, H.; Bosaeus, I.; Aman, P.; Stenling, R.; Dahlgren, S. Effect of Rye Bran on Excretion of Bile-Acids, Cholesterol, Nitrogen, and Fat in Human-Subjects with Ileostomies. *Am. J. Clin. Nutr.* **1994**, *59*, 389-394.
98. Jensen, M.K.; Koh-Banerjee, P.; Hu, F.B.; Franz, M.; Sampson, L.; Gronbaek, M.; Rimm, E.B. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *Am. J. Clin. Nutr.* **2004**, *80*, 1492-1499.
99. Andersson, M.; Ellegard, L.; Andersson, H. Oat bran stimulates bile acid synthesis within 8 h as measured by 7 alpha-hydroxy-4-cholesten-3-one. *Am. J. Clin. Nutr.* **2002**, *76*, 1111-1116.
100. Takahashi, R.; Hirasawa, Y.; Nichinari, K. Cellulose and its derivatives. *Foods and Food Ingr. J. Jap.* **2003**, *208*.

101. Prola, L.; Dobenecker, B.; Kienzle, E. Interaction between dietary cellulose content and food intake in cats. *J. Nutr.* **2006**, *136*, 1988s-1990s.
102. Dobenecker, B.; Kienzle, E. Interactions of cellulose content and diet composition with food intake and digestibility in dogs. *J. Nutr.* **1998**, *128*, 2674S-2675S.
103. Delorme, C.B.; Wojcik, J. Interaction of Dietary-Protein with Cellulose in the Adaptation to Caloric Dilution by Weanling Rats. *J. Nutr.* **1982**, *112*, 21-28.
104. Schwartz, S.E.; Levine, G.D. Effects of dietary fiber on intestinal glucose absorption and glucose tolerance in rats. *Gastroenterology* **1980**, *79*, 833-836.
105. Nelson, R.W.; Duesberg, C.A.; Ford, S.L.; Feldman, E.C.; Davenport, D.J.; Kiernan, C.; Neal, L. Effect of dietary insoluble fiber on control of glycemia in dogs with naturally acquired diabetes mellitus. *J. Am. Vet. Med. Assoc.* **1998**, *212*, 380-+.
106. Nelson, R.W.; Scott-Moncrieff, J.C.; Feldman, E.C.; DeVries-Concannon, S.E.; Kass, P.H.; Davenport, D.J.; Kiernan, C.T.; Neal, L.A. Effect of dietary insoluble fiber on control of glycemia in cats with naturally acquired diabetes mellitus. *J. Am. Vet. Med. Assoc.* **2000**, *216*, 1082-1088.
107. Nunes, C.S.; Malmlof, K. Effects of guar gum and cellulose on glucose absorption, hormonal release and hepatic metabolism in the pig. *Br. J. Nutr.* **1992**, *68*, 693-700.
108. Schwartz, S.E.; Levine, R.A.; Singh, A.; Scheidecker, J.R.; Track, N.S. Sustained pectin ingestion delays gastric emptying. *Gastroenterology* **1982**, *83*, 812-817.
109. Low, A.G.; Pittman, R.J.; Elliott, R.J. Gastric-Emptying of Barley Soybean Diets in the Pig - Effects of Feeding Level, Supplementary Maize Oil, Sucrose or Cellulose, and Water-Intake. *Brit J. Nutr.* **1985**, *54*, 437-447.
110. Takahashi, T.; Karita, S.; Ogawa, N.; Goto, M. Crystalline cellulose reduces plasma glucose concentrations and stimulates water absorption by increasing the digesta viscosity in rats. *J. Nutr.* **2005**, *135*, 2405-2410.
111. Lightowler, H.J.; Henry, C.J. Glycemic response of mashed potato containing high-viscocity hydroxypropylmethylcellulose. *Nutr. Res.* **2009**, *29*, 551-557.
112. Maki, K.C.; Davidson, M.H.; Witchger, M.S.; Dicklin, M.R.; Subbaiah, P.V. Effects of high-fiber oat and wheat cereals on postprandial glucose and lipid responses in healthy men. *Int. J. Vitam. Nutr. Res.* **2007**, *77*, 347-356.
113. Maki, K.C.; Davidson, M.H.; Torri, S.; Ingram, K.A.; O'Mullane, J.; Daggy, B.P.; Albrecht, H.H. High-molecular-weight hydroxypropylmethylcellulose taken with or between meals is hypocholesterolemic in adult men. *J. Nutr.* **2000**, *130*, 1705-1710.
114. Maki, K.C.; Carson, M.L.; Anderson, W.H.K.; Geohas, J.; Reeves, M.S.; Farmer, M.V.; Turowski, M.; Miller, M.; Kaden, V.N.; Dicklin, M.R.; Rains, T.M. Lipid-altering effects of different formulations of hydroxypropylmethylcellulose. *J. Clin. Lipidol.* **2009**, *3*, 159-166.
115. Higgins, J.A. Resistant starch: metabolic effects and potential health benefits. *J. AOAC Int.* **2004**, *87*, 761-768.
116. Haub, M.D.; Hubach, K.L.; Al-Tamimi, E.K. Different types of resistant starch elicit difference glucose responses in humans. *J. Nutr. Metab.* **2010**, *2010*, 4.
117. Behall, K.M.; Scholfield, D.J.; Hallfrisch, J.G.; Liljeberg-Elmstahl, H.G.M. Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women. *Diabetes Care* **2006**, *29*, 976-981.

118. Reader, D.M.; O'Connell, B.S.; Johnson, M.L.; Franz, M. Glycemic and insulinemic response of subjects with type 2 diabetes after consumption of three energy bars. *J. Am. Diet. Assoc.* **2002**, *102*, 1139-1142.
119. Al-Tamimi, E.K.; Sieb, P.A.; Snyder, B.; Haub, M.D. Consumption of Cross-Linked Resistant Starch (RS4XL) on Glucose and Insulin Responses in Humans. *J. Nutr. Metab.* **2009**, *2010*, 1-6.
120. Behall, K.M.; Scholfield, D.J.; Yuhaniak, I.; Canary, J. Diets Containing High Amylose Vs Amylopectin Starch - Effects on Metabolic Variables in Human-Subjects. *Am. J. Clin. Nutr.* **1989**, *49*, 337-344.
121. Reiser, S.; Powell, A.S.; Scholfield, D.J.; Panda, P.; Ellwood, K.C.; Canary, J.J. Blood lipids, lipoproteins, apoproteins, and uric acid in men fed diets containing fructose or high-amylose cornstarch. *Am. J. Clin. Nutr.* **1989**, *49*, 832-839.
122. Porikos, K.P.; Vanitallie, T.B. Diet-Induced Changes in Serum Transaminase and Triglyceride Levels in Healthy Adult Men - Role of Sucrose and Excess Calories. *Am. J. Med.* **1983**, *75*, 624-630.
123. Jenkins, D.J.A.; Vuksan, V.; Kendall, C.W.C.; Wursch, P.; Jeffcoat, R.; Waring, S.; Mehling, C.C.; Vidgen, E.; Augustin, L.S.A.; Wong, E. Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. *J. Am. Coll. Nutr.* **1998**, *17*, 609-616.
124. Tagliabue, A.; Raben, A.; Heijnen, M.L.; Deurenberg, P.; Pasquali, E.; Astrup, A. The Effect of Raw Potato Starch on Energy-Expenditure and Substrate Oxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 1070-1075.
125. Howe, J.C.; Rumpler, W.V.; Behall, K.M. Dietary starch composition and level of energy intake alter nutrient oxidation in "carbohydrate-sensitive" men. *J. Nutr.* **1996**, *126*, 2120-2129.
126. Robertson, M.D.; Bickerton, A.S.; Dennis, A.L.; Vidal, H.; Frayn, K.N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am. J. Clin. Nutr.* **2005**, *82*, 559-567.

References – Chapter 2

AACC. (1991) Approved Methods American Association of Cereal Chemists, St. Paul, MN.

ADA. (2000) Nutrition recommendations and principles for people with diabetes mellitus. Diabetes Care 23:S43-S46.

Akanji A.O., Bruce M.A., Frayn K.N. (1989) Effect of Acetate Infusion on Energy-Expenditure and Substrate Oxidation Rates in Non-Diabetic and Diabetic Subjects. European Journal of Clinical Nutrition 43:107-115.

Anugwa F.O.I., Varel V.H., Dickson J.S., Pond W.G., Krook L.P. (1989) Effects of Dietary Fiber and Protein-Concentration on Growth, Feed-Efficiency, Visceral Organ Weights and Large-Intestine Microbial-Populations of Swine. Journal of Nutrition 119:879-886.

Aronsson L., Huang Y., Parini P., Korach-Andre M., Hakansson J., Gustafsson J.A., Pettersson S., Arulampalam V., Rafter J. (2010) Decreased Fat Storage by Lactobacillus Paracasei Is Associated with Increased Levels of Angiopoietin-Like 4 Protein (ANGPTL4). Plos One 5.

Baer D.J., Rumpler W.V., Miles C.W., Fahey G.C. (1997) Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. Journal of Nutrition 127:579-586.

Barea R., Dubois S., Gilbert H., Sellier P., van Milgen J., Noblet J. (2010) Energy utilization in pigs selected for high and low residual feed intake. Journal of Animal Science 88:2062-2072.

Birketvedt G.S., Aaseth J., Florholmen J.R., Ryttig K. (2000) Long-term effect of fibre supplement and reduced energy intake on body weight and blood lipids in overweight subjects. Acta Medica (Hradec Kralove) 43:129-32.

Brighenti F. (1997) Simple method for quantitative analysis of short chain fatty acids in serum by gas-liquid chromatography, in: F. Guillon, et al. (Eds.), Plant polysaccharides in human nutrition: structure, function, digestive fate and metabolic affects, INRA, Nantes, France. pp. 114-9.

Brighenti F., Benini L., Del Rio D., Casiraghi C., Pellegrini N., Scazzina F., Jenkins D.J.A., Vantini I. (2006) Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. American Journal of Clinical Nutrition 83:817-822.

Brown M.B., Forsythe A.B. (1974) Robust Tests for Equality of Variances. Journal of the American Statistical Association 69:364-367.

Brownlee I.A., Moore C., Chatfield M., Richardson D.P., Ashby P., Kuznesof S.A., Jebb S.A., Seal C.J. (2010) Markers of cardiovascular risk are not changed by increased whole-grain intake: the WHOLEheart study, a randomised, controlled dietary intervention. British Journal of Nutrition 104:125-134.

Cummings S., Parham E.S., Strain G.W. (2002) Position of the American Dietetic Association: Weight management. Journal of the American Dietetic Association 102:1145-1155.

de Munter J.S.L., Hu F.B., Spiegelman D., Franz M., van Dam R.M. (2007) Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. Plos Medicine 4:1385-1395.

Du H.D., van der A D.L., Boshuizen H.C., Forouhi N.G., Wareham N.J., Halkjaer J., Tjonneland A., Overvad K., Jakobsen M.U., Boeing H., Buijsse B., Masala G., Palli D., Sorensen T.I.A., Saris W.H.M., Feskens E.J.M. (2010) Dietary fiber and subsequent changes in

body weight and waist circumference in European men and women. American Journal of Clinical Nutrition 91:329-336.
Fernandes J., Vogt J., Wolever T.M.S. (2011) Inulin increases short-term markers for colonic fermentation similarly in healthy and hyperinsulinaemic humans. European Journal of Clinical Nutrition 65:1279-1286.
Gao Z.G., Yin J., Zhang J., Ward R.E., Martin R.J., Lefevre M., Cefalu W.T., Ye J.P. (2009) Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. Diabetes 58:1509-1517.
Gardner C.D., Kiazand A., Alhassan S., Kim S., Stafford R.S., Balise R.R., Kraemer H.C., King A.C. (2007) Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. JAMA 297:969-77.
Grundy S.M., Brewer H.B., Cleeman J.I., Smith S.C., Lenfant C., Participants C. (2004) Definition of metabolic syndrome - Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. Circulation 109:433-438.
Haub M.D., Potteiger J.A., Jacobsen D.J., Nau K.L., Magee L.A., Comeau M.J. (1999) Glycogen replenishment and repeated maximal effort exercise: Effect of liquid carbohydrate. International Journal of Sport Nutrition 9:406-415.
Hedemann M.S., Eskildsen M., Laerke H.N., Pedersen C., Lindberg J.E., Laurinen P., Knudsen K.E.B. (2006) Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. Journal of Animal Science 84:1375-1386.
Heo J., Kattesh H.G., Roberts M.P., Morrow J.L., Dailey J.W., Saxton A.M. (2005) Hepatic corticosteroid-binding globulin (CBG) messenger RNA expression and plasma CBG concentrations in young pigs in response to heat and social stress. Journal of Animal Science 83:208-215.
Hong Y.H., Nishimura Y., Hishikawa D., Tsuzuki H., Miyahara H., Gotoh C., Choi K.C., Feng D.D., Chen C., Lee H.G., Katoh K., Roh S.G., Sasaki S. (2005) Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology 146:5092-5099.
Jenkins D.J., Wolever T.M., Leeds A.R., Gassull M.A., Haisman P., Dilawari J., Goff D.V., Metz G.L., Alberti K.G. (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Br Med J 1:1392-4.
Jenkins D.J.A., Wolever T.M.S., Jenkins A., Brighenti F., Vuksan V., Rao A.V., Cunnane S.C., Ocana A., Corey P., Vezina C., Connelly P., Buckley G., Patten R. (1991) Specific Types of Colonic Fermentation May Raise Low-Density-Lipoprotein-Cholesterol Concentrations. American Journal of Clinical Nutrition 54:141-147.
Jenkins D.J.A., Kendall C.W.C., Augustin L.S.A., Martini M.C., Axelsen M., Faulkner D., Vidgen E., Parker T., Lau H., Connelly P.W., Teitel J., Singer W., Vandenbroucke A.C., Leiter L.A., Josse R.G. (2002) Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. Diabetes Care 25:1522-1528.
Juntunen K.S., Laaksonen D.E., Poutanen K.S., Niskanen L.K., Mykkanen H.M. (2003) High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. American Journal of Clinical Nutrition 77:385-391.

Kelley D.E., Mandarino L.J. (2000) Fuel selection in human skeletal muscle in insulin resistance - A reexamination. Diabetes 49:677-683.
Koh-Banerjee P., Franz M.V., Sampson L., Liu S.M., Jacobs D.R., Spiegelman D., Willett W., Rimm E. (2004) Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. American Journal of Clinical Nutrition 80:1237-1245.
Koo S.H., Satoh H., Herzig S., Lee C.H., Hedrick S., Kulkarni R., Evans R.M., Olefsky J., Montminy M. (2004) PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nature Medicine 10:530-534.
Kornegay E.T. (1981) Soybean Hull Digestibility by Sows and Feeding Value for Growing-Finishing Swine. Journal of Animal Science 53:138-145.
Krauss R.M., Eckel R.H., Howard B., Appel L.J., Daniels S.R., Deckelbaum R.J., Erdman J.W., Kris-Etherton P., Goldberg I.J., Kotchen T.A., Lichtenstein A.H., Mitch W.E., Mullis R., Robinson K., Wylie-Rosett J., St Jeor S., Suttie J., Tribble D.L., Bazzarre T.L. (2000a) AHA dietary guidelines - Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Stroke 31:2751-2766.
Krauss R.M., Eckel R.H., Howard B., Appel L.J., Daniels S.R., Deckelbaum R.J., Erdman J.W., Jr., Kris-Etherton P., Goldberg I.J., Kotchen T.A., Lichtenstein A.H., Mitch W.E., Mullis R., Robinson K., Wylie-Rosett J., St Jeor S., Suttie J., Tribble D.L., Bazzarre T.L. (2000b) AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Stroke 31:2751-66.
Laakso M., Uusitupa M., Takala J., Majander H., Reijonen T., Penttila I. (1988) Effects of Hypocaloric Diet and Insulin Therapy on Metabolic Control and Mechanisms of Hyperglycemia in Obese Non-Insulin-Dependent Diabetic Subjects. Metabolism-Clinical and Experimental 37:1092-1100.
Lattimer J.M., Haub M.D. (2010) Effects of Dietary Fiber and its Components on Metabolic Health. Nutrients 2:1266-1289.
Lelliott C., Vidal-Puig A.J. (2004) Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. International Journal of Obesity 28:S22-S28.
Leone T.C., Lehman J.J., Finck B.N., Schaeffer P.J., Wende A.R., Boudina S., Courtois M., Wozniak D.F., Sambandam N., Bernal-Mizrachi C., Chen Z.J., Holloszy J.O., Medeiros D.M., Schmidt R.E., Saffitz J.E., Abel E.D., Semenkovich C.F., Kelly D.P. (2005) PGC-1 alpha deficiency causes multi-system energy metabolic derangements: Muscle dysfunction, abnormal weight control and hepatic steatosis. Plos Biology 3:672-687.
Lyon I., Masri M.S., Chaikoff I.L. (1952) Fasting and Hepatic Lipogenesis from C-14-Acetate. Journal of Biological Chemistry 196:25-32.
Marlett J.A., McBurney M.I., Slavin J.L. (2002) Position of the American Dietetic Association: health implications of dietary fiber. J Am Diet Assoc 102:993-1000.
Meyer K.A., Kushi L.H., Jacobs D.R., Slavin J., Sellers T.A., Folsom A.R. (2000) Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. American Journal of Clinical Nutrition 71:921-930.
Mokdad A.H., Ford E.S., Bowman B.A., Dietz W.H., Vinicor F., Bales V.S., Marks J.S. (2003) Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. Jama-Journal of the American Medical Association 289:76-79.
Murphy S.L., Xu J., Kochanek K.D. (2012) Deaths:Preliminary Data for 2010. National Vital Statistics Reports 60.

Nishina P.M., Freedland R.A. (1990) Effects of Propionate on Lipid Biosynthesis in Isolated Rat Hepatocytes. Journal of Nutrition 120:668-673.
NRC. (1996) Nutrient Requirements of Beef Cattle. 7th ed. National Academy Press, Washington, D.C.
NRC. (1998) (Ed.)^(Eds.) Nutrient Requirements of Swine: 10th Revised Edition, National Academy Press, Washington, D.C. pp. Pages.
Pereira M.A., Jacobs D.R., Pins J.J., Raatz S.K., Gross M.D., Slavin J.L., Seaquist E.R. (2002) Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. American Journal of Clinical Nutrition 75:848-855.
Petersen K.F., Dufour S., Savage D.B., Bilz S., Solomon G., Yonemitsu S., Cline G.W., Befroy D., Zemany L., Kahn B.B., Papademetris X., Rothman D.L., Shulman G.I. (2007) The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A 104:12587-94.
Pond W.G., Jung H.G., Varel V.H. (1988) Effect of Dietary Fiber on Young-Adult Genetically Lean, Obese and Contemporary Pigs - Body-Weight, Carcass Measurements, Organ Weights and Digesta Content. Journal of Animal Science 66:699-706.
Randle P.J. (1981) Citation Classic - the Glucose Fatty-Acid Cycle - Its Role in Insulin Sensitivity and the Metabolic Disturbances of Diabetes-Mellitus. Current Contents/Life Sciences:24-24.
Rigaud D., Ryttig K.R., Angel L.A., Apfelbaum M. (1990) Overweight treated with energy restriction and a dietary fibre supplement: a 6-month randomized, double-blind, placebo-controlled trial. Int J Obes 14:763-9.
Robertson M.D., Bickerton A.S., Dennis A.L., Vidal H., Frayn K.N. (2005) Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 82:559-67.
Samra R., Anderson G.H. (2007) Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men. American Journal of Clinical Nutrition 86:972-979.
Schneeman B.O. (1998) Dietary fiber and gastrointestinal function. Nutrition Research 18:625-632.
Schulze M.B., Liu S., Rimm E.B., Manson J.E., Willett W.C., Hu F.B. (2004) Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 80:348-56.
Schwartz A., Doucet E. (2010) Relative changes in resting energy expenditure during weight loss: a systematic review. Obesity Reviews 11:531-547.
Serena A., Jorgensen H., Knudsen K.E.B. (2009) Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. Journal of Animal Science 87:136-147.
Silva J.E. (2006) Thermogenic mechanisms and their hormonal regulation. Physiological Reviews 86:435-464.
Solum T.T., Ryttig K.R., Solum E., Larsen S. (1987) The influence of a high-fibre diet on body weight, serum lipids and blood pressure in slightly overweight persons. A randomized, double-blind, placebo-controlled investigation with diet and fibre tablets (DumoVital). Int J Obes 11 Suppl 1:67-71.
Tarini J., Wolever T.M.S. (2010) The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects.

Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme 35:9-16.
Ventura E., Davis J., Byrd-Williams C., Alexander K., McClain A., Lane C.J., Spruijt-Metz D., Weigensberg M., Goran M. (2009) Reduction in Risk Factors for Type 2 Diabetes Mellitus in Response to a Low-Sugar, High-Fiber Dietary Intervention in Overweight Latino Adolescents. Archives of Pediatrics & Adolescent Medicine 163:320-327.
Ville H., Maes G., Geers R., Goedseels V., Parduyns G., Vanbael J., Janssens S., Dekempeneer P. (1992) A Technique for Muscle Biopsy Sampling on Pigs to Assess Intramuscular Fat. Meat Science 32:123-129.
Washburn K.E., Powell J.G., Maxwell C.V., Kegley E.B., Johnson Z., Fakler T.M. (2005) A successful method of obtaining percutaneous liver biopsy samples of sufficient quantity for trace mineral analysis in adult swine without the aid of ultrasound. Journal of Swine Health and Production 13:126-130.
Weber T.E., Trabue S.L., Ziemer C.J., Kerr B.J. (2010) Evaluation of elevated dietary corn fiber from corn germ meal in growing female pigs. Journal of Animal Science 88:192-201.
Weickert M.O., Mohlig M., Schofl C., Arafat A.M., Otto B., Viehoff H., Koebnick C., Kohl A., Spranger J., Pfeiffer A.F.H. (2006) Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. Diabetes Care 29:775-780.
Wolever T.M.S., Spadafora P., Eshuis H. (1991) Interaction between Colonic Acetate and Propionate in Humans. American Journal of Clinical Nutrition 53:681-687.
Wolever T.M.S., Spadafora P.J., Cunnane S.C., Pencharz P.B. (1995) Propionate Inhibits Incorporation of Colonic [1,2-C-13]Acetate into Plasma-Lipids in Humans. American Journal of Clinical Nutrition 61:1241-1247.
Wolever T.M.S., Brighenti F., Royall D., Jenkins A.L., Jenkins D.J.A. (1989) Effect of Rectal Infusion of Short Chain Fatty-Acids in Human-Subjects. American Journal of Gastroenterology 84:1027-1033.
Wolever T.M.S., Schrade K.B., Vogt J.A., Tsihlias E.B., McBurney M.I. (2002) Do colonic short-chain fatty acids contribute to the long-term adaptation of blood lipids in subjects with type 2 diabetes consuming a high-fiber diet? American Journal of Clinical Nutrition 75:1023-1030.
Yen J.T., Varel V.H., Nienaber J.A. (2004) Metabolic and microbial responses in western crossbred and Meishan growing pigs fed a high-fiber diet. Journal of Animal Science 82:1740-1755.
Zhu P., Goh Y.Y., Chin H.F.A., Kersten S., Tan N.S. (2012) Angiopoietin-like 4: a decade of research. Bioscience Reports 32:211-219.

Appendix A - IACUC Approval

K-STATE
Kansas State University
University Research
Compliance Office
203 Fairchild Hall
Lower Mezzanine
Manhattan, KS 66506-1103
785-532-3224
Fax: 785-532-3278
www.k-state.edu/research/comply

TO: Jim Nelssen
ASI
243 Weber Hall

Protocol Number: 2983

FROM: Sally Olson, Chair
Institutional Animal Care and Use Committee

DATE OF APPROVAL: May 10, 2011
DATE OF EXPIRATION: May 10, 2014

RE: Approval of Animal Care and Use Protocol Entitled, "Effect of soluble and insoluble dietary fiber on hepatic and myocellular markers of lipid metabolism."

The Institutional Animal Care and Use Committee (IACUC) for Kansas State University has reviewed the protocol identified above and has approved it for three years from the date of this memo. During the period of approval, the protocol will be subject to annual monitoring, which may include the examination of records connected with the project. Announced post-approval monitoring (PAM) will be performed during the course of this approval period by a member of the University Research Compliance Office staff. Changes in the protocol affecting the care or use of animals must be reviewed by the IACUC prior to implementation. Unanticipated problems related to the humane care or use of animals must be reported to the IACUC immediately.

It is important that your animal care and use project is consistent with submissions to funding/contract entities. It is your responsibility to initiate notification procedures to any funding/contract entity of any changes in your project that affects the use of animals.