MODULATION OF BIO-BEHAVIORS AND DIABESITY
BY DIETARY FACTORS

BY
MELISSA M. KACZMARCZYK

DISSERTATION
Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Nutritional Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

Doctoral Committee:
Professor Emeritus George Fahey, Chair
Professor Gregory Freund, Director of Research
Assistant Professor Michael Miller, Co-Director of Research
Assistant Professor Ryan Dilger
Obesity is associated with a host of co-morbidities including hyperglycemia, insulin resistance, Type II diabetes (T2D), and cognitive deficit. The present study examined the modulation of weight, blood metabolites, bio-behaviors, and neuro-immune function by a high fat diet. C57BL/6J male mice were fed a 60% fat diet (high fat diet; HFD) or a 10% fat (low fat diet; LFD) for 1 or 3 weeks post-weaning, after which mice were subjected to behavioral testing. HFD-fed mice had increased anxiotal-like and reward seeking behavior and decreased performance in spatial and non-spatial memory tasks compared to mice fed a LFD. There was no difference in spontaneous locomotion or depressive-like behavior between diet groups. Additionally, non-spatial memory deficit was restored in mice fed HFD for 1 week by feeding a LFD for 1 week. Interestingly, IL-1R1 -/- mice displayed non-spatial memory deficit regardless of diet, implicating a role for IL-1 in HFD-induced memory deficit. Analysis of the mRNA expression of key cytokines and growth factors in the hippocampus, hypothalamus, and cortex did not show differences in the expression of IL-1 or IL-1 receptors after 1 week of diet feeding, but showed reduced expression of two key growth factors related to memory, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), in HFD-fed mice compared to LFD-fed mice. Finally, memory impairment in HFD-fed mice was corrected with administration of methylphendidate, a stimulant that increases the synaptic concentration of dopamine and norepinephrine by blocking dopamine transporters. Anti-depressants, desipramine and reboxetine, both norepinephrine re-uptake inhibitors, did not correct memory deficit in HFD-fed mice. Dietary fiber (DF) is associated with a reduction in the risk of obesity and T2D. The present study also examined the protective effect of soluble and insoluble fibers on weight gain and T2D. C57BL/6J male mice were fed a HFD or LFD supplemented with pectin, cellulose, resistant starch, or fructooligosaccharide for 10-12 weeks. Mice fed a HFD + 10% pectin were protected against weight gain, while mice fed a HFD + 5% pectin, 5% pectin and 5% cellulose, 10% resistant starch, or 10% fructooligosaccharides were not. Supplementation of a HFD with 10% pectin also slowed HFD-induced elevation of fasting blood glucose and significantly
decreased blood glucose concentrations when presented with an intraperitoneal glucose challenge. Supplementation of a HFD with 10% pectin slowed weight gain in IL1R1-/-, IL4-/-, and MyD88-/- mice, but not in TLR4-/- or TLR2-/- mice. Finally, fecal lipid excretion was significantly increased in pectin-supplemented animals compared to cellulose, suggesting that pectin’s ability to form viscous gels results in decreased lipid digestion and absorption, contributing to the differences noted in weight gain between diet groups. This research demonstrates that HFD-feeding has an immediate, detrimental effect on bio-behaviors in juvenile mice. In addition, this research demonstrates that HFD-induced weight gain and hyperglycemia may be delayed by pectin supplementation.
ACKNOWLEDGMENTS

I would like to thank everyone who supported me in this journey. You know who you are.

Well behaved women seldom make history- Laurel Thatcher Ulrich
## TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1 Significance
1.2 Specific Aims
1.3 References

CHAPTER 2: LITERATURE REVIEW: THE HEALTH BENEFITS OF DIETARY FIBER: IS THERE ANYTHING BEYOND THE DISCUSSION OF DIABESITY, CARDIOVASCULAR DISEASE, AND COLON CANCER?

2.1 Introduction
2.2 Defining Dietary Fiber
2.3 Diabesity
2.4 Inflammation
2.5 Cognition and Memory
2.6 Conclusion
2.7 Tables
2.8 References

CHAPTER 3: THE MODULATION OF BIO-BEHAVIORS BY A SHORT-COURSE HIGH FAT DIET IN JUVENILLE MICE

3.1 Introduction
3.2 Methods
3.3 Results
3.4 Discussion
3.5 Figures
3.6 References

CHAPTER 4: MODULATION OF OUTCOMES PERTAINING TO DIABESITY BY DIETARY FIBER

4.1 Introduction
4.2 Methods
4.3 Results
4.4 Discussion
4.5 Tables and Figures
4.6 References

CHAPTER 5: SUMMARY AND DISCUSSION
CHAPTER 1
Introduction

1.1 Significance

The prevalence of juvenile obesity has more than tripled in the last 30 years (1). Especially concerning is the accompanying rise in previously “adult-only” co-morbidities such as hypertension, hyperglycemia, insulin resistance, Type II diabetes, cardiovascular disease, and stroke (1-3). Furthermore, increasing epidemiological evidence suggests an association between obesity and reduced cognitive function and psychiatric disorders such as attention deficit hyperactivity disorder (ADHD) and dementia (4-7). The prevalence of ADHD has been found to be higher in obese cohorts compared to non-obese controls (8, 9). Additionally, obesity and ADHD share similar pathophysiology, such as anxiety, inattention, and executive function deficit (10, 11). There is some debate as to whether inflammation associated with obesity leads to cognitive dysfunction noted in ADHD or if increased impulsivity and poor decision making ability lead to obesity (8). A third possibility is that both ADHD and obesity share the same mechanism involving the dopaminergic pathway (11, 12). As the consumption of fast food and convenience food increased globally, the global prevalence of juvenile obesity has increased exponentially. Understanding the effects of short-term exposure to a high fat diet (HFD) on bio-behaviors such as spontaneous movement, learning, and anxieta/depressive-like behaviors in juveniles is crucial, as these are critical in early brain development.

The current study focused on the immediate effect of a HFD on modulation of bio-behaviors related to both obesity and ADHD: anxiety, depression, locomotion, and cognition. In addition, this study further explored the proposed common mechanism between these conditions by examining HFD-induced changes in cytokines and growth factors purported to alter the dopaminergic pathway.

Dietary fiber (DF) has been associated with risk reduction of obesity and several related co-morbidities such as type II diabetes (T2D) and cardiovascular disease (13-16). The health benefits of DF are dependent on the type of DF and the physical characteristics of the fiber. Benefit is conferred in part by altering gastrointestinal transit, slowing gastric emptying, and by
delaying or inhibiting the absorption of macronutrients. Degree of solubility, fermentability, and gel-forming ability varies by DF. This is reflected in the inconsistent results of clinical trials exploring the ability of DF to reduce weight (14, 17-20) and hyperglycemia (21, 22). Understanding the attributes of each fiber is critical in developing dietary interventions. This study also explored DF supplementation of a HFD. Here, DF of varying solubility and fermentability were used to determine extent of protection against weight gain. Additionally, pectin was used to further explore the mechanism by which soluble fiber reduces the risk of diabesity (obesity + T2D).

This study contributes novel and critical research to understanding the role of a HFD on the co-morbid conditions of obesity and ADHD. It also provides additional insight into the beneficial effects of DF on prevention of obesity and T2D.

### 1.2 Specific Aims

**Specific Aim 1:**
Determine the immediate effect of a HFD on bio-behaviors and neuro-immune function in a juvenile mouse model.

We hypothesized that HFD feeding would increase anxiety, depression, and memory deficit in mice after 3 weeks of feeding. Additionally, we hypothesized that HFD feeding would result in disregulation of pro-inflammatory cytokines and growth factors related to neuronal plasticity, specifically in the hippocampus and cortex, areas of the brain that are related to memory and learning.

**Specific Aim 2:**
Determine the effect of dietary fiber supplementation of HFD on weight modulation and blood metabolites related to type II diabetes.

We hypothesize that soluble fibers (pectin, fructooligosaccarides, and resistant starch) would result in less weight gain over a 12 week period than the insoluble fiber, cellulose. In addition,
we predicted that pectin would reduce hyperglycemia in HFD-fed animals compared to cellulose-fed animals.

Secondary Aim 2:
Determine if the ability of pectin to prevent weight gain is solely due to the capacity of pectin to form viscous gels.

We hypothesized that pectin-fed animals would have increased fecal lipid excretion compared to cellulose-fed animals. We also hypothesized that knockout animals deficient in TLR4, TLR2, and MyD88 would not be protected from HFD-induced weight gain by pectin supplementation due to lack of receptor function.
### 1.3 References


CHAPTER 2
Literature Review:
The health benefits of dietary fiber: Is there anything beyond the discussion of diabesity, cardiovascular disease, and colon cancer?

2.1 Introduction
Dietary fiber (DF) decreases the risk of type 2 diabetes (T2D), cardiovascular disease, and colon cancer (1-4) by reducing the digestion and absorption of macronutrients and by decreasing contact time of carcinogens with the intestinal lumen (1, 2, 5, 6). In addition, health claims supporting the role of DF in the prevention of cancer and coronary heart disease (CHD) have been approved by the Food and Drug Administration in the United States (7, 8). More recently, and perhaps more interestingly, epidemiological studies have found the benefits of DF to extend beyond T2D, CHD, and colon cancer. A 9 year follow-up study of 567,169 American Association of Retired Persons (AARP) members aged 50-71 years old found that in addition to reducing the risk of all-causes of mortality, intake of DF was inversely related to mortality from respiratory and infectious diseases (9). In a cross-sectional study of 1538 pregnant women, higher DF intake was associated with reduced risk of preeclampsia (10). DF also may increase positive mood, cognition, and alertness (11, 12). The mechanisms by which DF specifically exerts all its protective effects remain to be elucidated, but its ability to physically disrupt macronutrient absorption has been extensively explored. More recently, the role of DFs in positively impacting human health through alteration of the intestinal microbiome has gained significant traction (13, 14). DF-dependent changes in gut bacterial composition are thought to modify host metabolism in a variety of ways ranging from the enhancement of bile acid deconjugation (15, 16), production of short chain fatty acids (SCFAs) (17), to modulation of inflammatory bioactives. Here we define DF and provide an overview of the impact of DF on metabolism and the microbiome. We also discuss the impact of DF on conditions and diseases not directly linked to diabesity, CHD, and colon cancer.

2.2 Defining Dietary Fiber
DF is a broad category of non-digestible food ingredients that include non-starch polysaccharides, oligosaccharides, lignin, and analogous polysaccharides that have an associated
health benefit (3, 6). The physical properties of DFs vary and even a slight variance may influence the physiological effect of the DF. Generally, DFs are classified by solubility in water, microbial fermentation in the large intestine, and viscosity. Soluble DFs include pectin, gums, and polysaccharides, while insoluble DFs include cellulose, hemicelluloses, and lignin (3).

Previously, it was erroneously believed that soluble DFs decreased serum lipids and cholesterol while insoluble DFs solely contributed to fecal bulking. Current thought attributes the physiologic effects of a particular DF to its degree of viscosity and fermentation (18). In addition, the definition of what comprises a DF has been expanded to include resistant starches and oligosaccharides that have properties similar to soluble DFs (19). Prebiotic DFs are not classified in terms of solubility or viscosity but are defined by resistance to digestion and absorption in the small intestine, partial or complete fermentation by microbiota in the large intestine, and by the ability to stimulate growth of select bacteria (17). Only two such DFs fit all of the above three criteria and are considered prebiotics: inulin and trans-galactooligosaccharides. In the near future, polydextrose and several other oligosaccharides may be classified as prebiotics due to their ability to meet the above conditions (20).

The human microbiome is dominated by Bacteroides and Firmicutes (21). Fermentable DFs shift gut microbial populations by providing substrates for select bacteria to ferment. Fructooligosaccharides and galactooligosaccharides increase fecal bifidobacteria and lactobacillus populations in infants (22, 23), while inulin increases populations of bifidobacteria in adults (24). DFs can be partially or completely fermented to the SCFAs acetate, propionate, and butyrate (25) (Table 1). Fermentation patterns and the ratio of SCFAs produced are dependent on DF type. Acetate is the main product of pectin fermentation (26), while fermentation of gum arabic and dextrins results primarily in propionate (27, 28). Okara increased cecal butyrate and decreased cecal acetate concentrations in female rats fed 10% okara for 4 weeks compared to standard rodent chow (29). Acetate and propionate enter the liver via portal circulation where they are almost fully metabolized (30). Butyrate, on the other hand, is metabolized by β-oxidation in colonic enterocytes (31). Finally, the physiologic effects of DFs are due, in part, to type and amount of SCFAs produced and the ability of these SCFAs to stimulate intestinal water and sodium absorption as well as influence gut pH and bile salt precipitation (32).
2.3 Diabesity

Given the linkage between elevated body mass index (BMI), T2D, and CHD (2, 19), the role of DF in weight reduction has been examined in animal and human studies. A strong inverse relationship between DF intake and weight has been established in animal models (28, 29, 33-36) and by epidemiological studies (37-40). A prospective cohort study of 74,091 females spanning 12 years found women in the highest quintile for whole grain consumption had a 49% lower risk of major weight gain than women in the lowest quintile. Interestingly, the effect of DF on prevention of weight gain was most significant in individuals who were overweight at baseline (39). A prospective cohort study of 894,332 European individuals followed for 6.5 years found total DF intake associated with weight and waist circumference (37). In this study, cereal DF was associated with weight while DF from fruit and vegetables was not. Unfortunately, research tying increased DF intake to weight loss is inconsistent in human clinical trials (3). Overall, conflicting results in humans may be due to DF type, dosage, and study population. A meta-analysis consisting of 14 random control trials, examining glucomannan and weight loss, found that glucomannan supplementation significantly reduced weight (41). In a randomized placebo controlled study of 167 overweight individuals, supplementation of 1240 mg/day of glucomannan in addition to a calorie-restricted diet resulted in greater weight loss after 5 weeks than a calorie-restricted diet alone (42). In a parallel double blinded placebo-controlled clinical trial, weight loss tended to be greater, but not significantly so, in obese individuals receiving supplements of 3 grams Plantago ovarto (psyllium) + 1 gram glucomannan 2 or 3 times per day in addition to a calorie-restricted diet for 16 weeks compared to placebo (43). While glucomannan seemed to show promise in the reduction of weight, not all clinical trials have demonstrated a weight loss effect. Supplementation of 10 grams of guar gum (glucomannan) 3 times per day for 6 weeks did not result in a weight difference compared to the control period in a double blind placebo-controlled cross-over trial of 25 non-obese men. Overall, diet supplementation with DF appears to be more effective for weight reduction in overweight or obese populations and when used in combination with a calorie-restricted diet. Clinical interventions with other DFs also have produced inconsistent results regarding weight (3). As an example, chitosan, a DF popular in over-the-counter weight loss treatments, reduced weight in a random double blind placebo-controlled clinical trial of 230 overweight or obese persons receiving 3 grams chitosan or a placebo daily for 24 weeks (44). While the weight loss in this
study was significant between the DF and placebo groups (0.4% loss in the chitosan group compared to a 0.2% gain in the placebo group), the reduction in weight was not clinically relevant. In sum, a diet high in a variety of soluble and insoluble DFs from whole grains, cereals, fruits, and vegetables may be more effective in weight regulation than supplementation with a single DF.

Reduction in T2D risk by DFs also appears to be dependent on type and dose of DF and study population. In animals, soluble DFs decrease T2D related bio-markers. In mice, 10% psyllium and 10% sugar cane fiber decreased fasting blood glucose and fasting plasma insulin when added to a high fat diet for 12 weeks when compared to the insoluble fiber, cellulose (45). β-glucan also improved glucose tolerance and decreased serum insulin in mice when added to a high fat diet at a 2 and 4% level (34). In contrast, diabetic dogs fed an insoluble DF had lower maximum and mean blood glucose concentrations and a lower area under the blood glucose curve compared to dogs fed a soluble DF or a low DF diet (46). In humans, muffins high in β-glucan and resistant starch lowered postprandial blood glucose and insulin more effectively than muffins containing low or medium β-glucan/resistant starch (5). Plantago ovarta husk supplementation of 14 grams per day for 8 weeks, however, reduced serum insulin but not plasma glucose compared to placebo (47). Surprisingly, despite the ability of soluble DFs to reduce diabetes-associated biomarkers, soluble DFs have not been associated with a reduced risk of T2D in population-based studies (48). In a large prospective cohort study of 99,826 women, intake of total DF, total insoluble DF, and DF from cereal was inversely related to risk of diabetes. There was no association found between intake of soluble DF and diabetes risk (48). This again highlights the difference in effectiveness between DFs as part of a balanced diet and DFs as stand-alone supplements.

The protective effect of DF on obesity and T2D has historically been attributed to greater satiety resulting from increased mastication, calorie displacement, and decreased absorption of macronutrients (49). The mechanism is believed to be due to the ability of soluble DFs to form viscous solutions that prolong gastric emptying, thus inhibiting the transport of glucose, triglycerides, and cholesterol across the intestinal wall (6, 46, 50-52). As an example guar gum was shown to reduce in vitro glucose absorption by reducing simulated intestinal contractions
Viscous DFs form gels that decrease luminal content contact with pancreatic lipases and bile by forming DF-lipid aggregates that block fat emulsification and micelle formation (51). Increased viscosity of luminal contents also thickens the unstirred water layer, thereby decreasing diffusion and uptake of cholesterol and glucose from the intestinal lumen (54). Certain DFs can, in turn, bind bile acids and micelle components such as monoglycerides, free fatty acids, and cholesterol that decrease absorption and increase fecal excretion of these entities (55-57).

DFs also modify lipid (Table 2) and carbohydrate (Table 3) metabolism by influencing the expression of key genes and hormones. Acetyl-CoA carboxylase is the rate limiting enzyme in lipogenesis and is regulated by AMP-activated protein kinase. In a 10 week study comparing obese and lean rats, 5 grams of Plantago ovarto, when added to rat chow, increased the phosphorylation of AMP-activated protein kinase, thus inhibiting acetyl-CoA carboxylase. This inhibition of acetyl-CoA carboxylase in obese rats was comparable to the acetyl-CoA carboxylase activity in lean rats fed a control diet (58). Fructooligosaccharide (10 grams/100 grams) also has been shown to decrease hepatic acetyl-CoA carboxylase expression in rats (59). Increases in SCFAs due to bacterial fermentation may activate hepatic AMP-activated protein kinase (60). Synthesis of fatty acids, primarily palmitate, is catalyzed by the fatty acid synthase complex. Reductions in fatty acid synthase expression have been demonstrated in rodents fed resistant starch, fructans, inulin, β-glucan, Plantago ovarto, and hydroxylpropylmethycellulose (34, 35, 58, 59). Alteration in gene expression may be due to DF-induced modulation of gut microbiota and subsequent SCFA production. Conventionalization of germ free mice resulted in increased hepatic triglycerides with a corresponding increase in mRNA expression of hepatic acetyl CoA carboxylase-1 and fatty acid synthase (61). Butyrate activates colonic fatty acid synthesis by contributing carbon atoms for de novo lipogenesis (62, 63). Finally, supplementation of 1.0% gum arabic in the drinking water of female mice over 180 days resulted in increased expression of triacylglycerol lipase and hormone sensitive lipase in paranephric fat, enzymes involved in mobilizing fatty acids from adipocytes (28, 64). Gum arabic also increased expression of fasting-induced adipose factor, a lipoprotein lipase inhibitor that blocks the incorporation of fatty acids into low density lipoprotein (28). This increase in fasting-induced
Adipose factor may be the result of modulation of the intestinal microbiome as fasting-induced adipose factor is synthesized in the intestinal epithelium in response to microbiota (61).

The rate-limiting enzyme of cholesterol synthesis, HMG-CoA reductase, converts HMG-CoA to mevalonate. While β-glucan supplementation in mice did not alter hepatic expression of HMG-CoA reductase, hydroxylpropylmethylcellulose was shown to increase hepatic expression in hamsters (34, 35). A combination of inulin and oligofructose, however, increased hepatic expression of HMG-CoA reductase in rats (65). Increased HMG-CoA reductase may be due to a depletion of cholesterol pools resulting from increased excretion of bile cholesterol. Catabolism by microbial populations also may be important. Bacteria such as L. acidophilus and bifidobacteria can exert a hypocholesterolemic effect by enhancing bile acid deconjugation (15, 16). Furthermore, lactobacilli and bifidobacteria remove cholesterol in vitro by assimilation and precipitation (25, 66). Fermentation products further affect lipid metabolism. Propionate inhibits the incorporation of acetic acid into fats and sterols, resulting in decreased fatty acid and cholesterol synthesis. Demigne et al. demonstrated, in rats, that propionate inhibited fatty acid and cholesterol synthesis by blocking the integration of C1,4 into fatty acids (62).

Carbohydrate metabolism also is influenced by DF intake. Insoluble DFs appear to improve insulin sensitivity, but the mechanisms by which insoluble DFs impact insulin signaling is unclear (6, 67). Both soluble and insoluble DFs may be involved in the regulation of hormones such as glucose-dependent insulin tropic polypeptide and glucagon-like peptide-1 that stimulate postprandial insulin release, enhance glucose tolerance, and delay gastric emptying (2, 68). In a study of 14 women, control meals of insoluble DF increased insulin responsiveness as well as glucose-dependent insulin tropic peptide (67). Proglucagon, a precursor of glucagon-like peptide 1, is produced in the L-cells of the distal ileum and colon and is increased by butyrate production (69). A diet high in fermentable DFs, as opposed to cellulose, increased expression of proglucagon mRNA in the ileum of dogs and rats and the colon of dogs (36, 69). An increase in postprandial plasma glucagon-like peptide-1 was noted as well (6, 69). DFs may increase proglucagon and glucagon-like peptide-1 by augmenting the number of L-cells in the intestine (13).
Shifts in the gut microbial population affect the manifestation of diabesity. As noted above, the human microbiome is comprised primarily of bifidobacteria and firmicutes (21). Obesity in both mice and humans is linked to an increase in firmicutes and a decrease in bifidobacteria (21). This microbial misbalance is corrected by weight loss in humans. The obesity-associated microbiome has a heightened capacity for energy harvest (70), which may attenuate weight gain. Certain DFs, such as inulin and oligofructose, increase bifidobacteria populations and may prevent a shift towards an obesity-associated microbiome (71, 72). As evidence, antibiotic-induced changes in gut microbial populations of obese mice resulted in improved glucose tolerance (73), indicating that the obese state microbiome also may influence diabetes related characteristics.

2.4 Inflammation

Chronic inflammation is inherent to a variety of diseases, including diabesity. DFs appear to be anti-inflammatory, decreasing inflammation-associated biomarkers and bioactives including C-reactive protein (CRP), IL-6, and TNF-α (58, 74, 75). In a case-control study of 88 individuals that examined the relationship of DF intake to plasma cytokine and chemokine concentrations, no association was found between the intake of total, soluble, or insoluble DF to any cytokine or chemokine examined. However, intake of cereal DF was associated with a reduction in an inflammatory bioactive array that was comprised of IL-1β, IL-6, TNF-α, IL-4, IL-5, and IL-13 (76). This immune profile suggests anti-inflammation seen with the macrophage deactivation (IL-10) phenotype. Conversely, in a cross-sectional study of 1953 overweight post-menopausal women, intake of total, soluble, and insoluble DFs were all inversely associated with plasma IL-6 and TNF-α concentrations (75). Several epidemiologic studies have demonstrated an inverse relationship between DF intake and CRP, a sensitive marker of inflammation, and CHD (74, 77, 78). In an examination of NHANES data containing 14,533 adults, intake of total, insoluble, and soluble DFs were linked to lower serum CRP. In addition, a subgroup of persons with chronic kidney disease who reported higher DF intake had decreased mortality from all causes (78). While most epidemiologic studies demonstrate an association between DF intake and anti-inflammatory properties, cohort and cross-sectional study designs make it difficult to ascertain if DF supplementation would be successful in reducing inflammation. To this end, intervention trials have reported inconsistent results which, as mentioned earlier, may reflect
fiber type, dose, and study population. In a prospective randomized controlled trial of 158 overweight or obese persons receiving 7 or 14 grams per day of psyllium, there was no difference in serum CRP or IL-6 concentrations compared to the control group (79). Distinctly, in a pre-test/post-test study involving 19 elderly nursing home patients, 4 grams of fructooligosaccharides delivered 2 times per day for 3 weeks caused a decrease in granulocyte and monocyte phagocytic activity and lower blood IL-6. This study additionally demonstrated increased fecal bifidobacteria (72). Supplementation of fructooligosaccharides in infant formula, however, appears to afford long-lasting protection against allergens and infection. Galactooligosaccharides and fructooligosaccharides added to the formula of 134 infants for 6 months resulted in a lower incidence of atopic dermatitis, wheezing, upper respiratory tract infections, and fever in the 2 years following intervention (80). Lending support to the relevance of galactooligosaccharides to infection prevention is a study in 427 college students given 2.5 or 5 grams of galactooligosaccharide daily. Over the 8 week study period when compared to control subjects, stress-induced gastrointestinal symptoms were decreased as were the percentage of days of cold and flu (81). Given that fructooligosaccharides and galactooligosaccharides increase fecal bifidobacteria and lactobacilli (22, 23, 72), the immunomodulation seen in the above human studies may be the result of DF-induced changes in gut microbial populations.

With fermentable DFs, gut-generated SCFAs may be responsible for the modulation of appetite, insulin signaling, and inflammation. In adipose tissue, propionate down-regulated the pro-inflammatory cytokine TNF-α (82). In vitro, SCFAs suppressed release of TNF-α from LPS-stimulated human neutrophils while also reducing IL-6 mRNA and protein in cultures of human colonocytes (83). SCFAs appear to communicate with the immune system through G-protein-coupled free fatty acid receptors. Free fatty acid receptor 3 (FFA3) is expressed primarily by adipocytes and is activated by propionate, butyrate, and acetate. Activation of adipocyte FFA3 by propionate leads to elevated leptin secretion, while activation by butyrate sparks adipogenesis (84). FFA2 expression is noted in leukocytes and colonic L-cells in addition to adipocytes. FFA2 is activated primarily by propionate (84) and to a lesser extent by acetate (85). Like FFA3, FFA2 may regulate differentiation of adipocytes but it also appears to lessen fat cell triglyceride storage capacity and accumulation of body fat deposits (86). How this relates to the ability of FFA2 to decrease plasma free fatty acids (84) and increase adipocyte insulin sensitivity is unclear (86).
Interestingly, DFs may increase cellular expression of FFA2 as 5% nopal (a DF containing a 40:60 ratio of soluble to insoluble fiber) increased colonic FFA2 by 31% in rats (87). Bypassing FFA2, DFs may interact directly with immunoregulatory cells. Mucosal macrophages and dendritic cells have pattern recognition receptors with carbohydrate binding domains that are able to bind certain DFs, such as β-glucans, causing decreased IL-12 and increased IL-10 (88) which is consistent with an anti-inflammatory phenotype. Overall, the mechanism by which fermentable DFs work may not be principally related to their ability to act as a SCFA source.

2.5 Cognition and Memory

Inflammation is an important component of many neurodegenerative diseases. Alzheimer’s disease and vascular dementia are both associated with increased CRP, IL-6, and TNF-α (89). CRP is neurotoxic and neuron-synthesized CRP can initiate neuro-apoptosis (89). The progression of Alzheimer’s disease has been linked to both oxidative stress and AB-induced neurotoxicity. An extract of *Triticum aestivium* L., a type of wheat crop, was shown to protect cells from AB cytotoxicity and apoptosis in vitro. Additionally, *Triticum aestivium* L. reversed scopolamine-induced spatial memory deficits in rats (90). While research in this area is limited, DF intake may affect cognition and mood due to the role of systemic inflammation in depressive and anxietaal disorders (89). A 1.5 g fiber breakfast bar increased positive mood and performance on a free recall task (12). DF also has been shown to ameliorate pre-depression-associated sickness behaviors in mice because diets containing 10% pectin reduced endotoxin-dependent social withdrawal and fever compared to diets containing 10% cellulose (91). Furthermore, in a community-based study of 394 post-menopausal women, ingestion of lignan improved cognitive performance (11). Mechanistically, microbiota-dependent production of SCFAs is likely key to DF-induced memory and behavioral changes because administration of SCFAs elicit alterations in brain functions. Butyrate, possibly via histone hyperacetylation, increased brain-derived neurotrophic factor, a bioactive critical to neuronal plasticity (92). Administration of propionate to rats induced autism-like behavioral changes (92), while diet-induced alterations in gut microbiota generated detrimental effects on mouse cognition and behavior (93). Finally, dysregulation within the hypothalamic-pituitary-adrenal (HPA) axis results in depression and
anxiety (92) and commensal bacteria have been shown to influence the HPA axis and reduce behavioral abnormalities (92).

2.6 Conclusion

The definition and chemical characterizations of DFs are well documented and understood as is the ability of DFs to reduce absorption of macronutrients by increasing viscosity of luminal contents and altering intestinal transit time. The impact of DFs on lipid metabolism is clearly delineated, especially in terms of enhancing expression of key enzymes of β-oxidation and de novo lipogenesis, namely triacylglycerol lipase, hormone-sensitive lipase, acetyl-CoA carboxylase, and fatty acid synthetase. DFs are hypocholesterolemic, modulating expression of HMGCoA reductase, decreasing cholesterol synthesis, and increasing excretion of cholesterol in bile. The process by which DFs improve glycemic control and insulin sensitivity are less clear. Soluble DFs may lower blood glucose by slowing carbohydrate absorption in the gut via increased viscosity. Epidemiological studies, however, fail to find a solid relationship between soluble DF intake and reduced risk of T2D. Finally, emerging research suggests that DFs may influence immunity by altering plasma concentrations of key bioactives such as CRP and TNF-α, through modulation of gut microbiota. Such immune system modifications likely tied to SCFA and FFA2 suggest a link to the brain-cytokine system as evidenced by the ability of certain DFs to regulate sickness symptoms and cognitive function. While these exciting new functions for DFs are intriguing, much work needs to be done to determine the mechanism by which DFs exert these phenomena.
### 2.7 Tables

<table>
<thead>
<tr>
<th>Fiber</th>
<th>Species</th>
<th>Propionate</th>
<th>Acetate</th>
<th>Butyrate</th>
<th>Total SCFA</th>
<th>Fecal Lipid</th>
<th>Fecal Bile</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Cyclodextrin</td>
<td>rodent</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>β-Cyclodextrin</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>49</td>
</tr>
<tr>
<td><em>Cassia tora</em> Linn.</td>
<td>rodent</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>Hydroxypropyl-methylcellulose</td>
<td>rodent</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>rodent</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Okara</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Pectin</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>41.67</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>rodent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>36</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>rodent</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

*"+" indicates dietary fiber affects this property positively or negatively. "-" indicates dietary fiber did not have an effect on this property.*
Table 2. Summary of the effects of fiber on biomarkers related to dyslipidemia and hypercholesterolemia

<table>
<thead>
<tr>
<th>Fiber</th>
<th>Species</th>
<th>Serum Lipids</th>
<th>Hepatic Lipids</th>
<th>Free Fatty Acids</th>
<th>Plasma TG</th>
<th>Hepatic TG</th>
<th>Total Plasma Cholesterol</th>
<th>Hepatic Cholesterol</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Cyclodextrin</td>
<td>rodent</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>β-Cyclodextrin</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td></td>
<td></td>
<td>49</td>
</tr>
<tr>
<td>β-glucan (barley)</td>
<td>rodent</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td>14,40</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>-</td>
<td></td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Blueberry peel extract</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>Cassia tora Linn.</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
<td>16,92</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td></td>
<td></td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>rodent</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Glucomannan (guar gum)</td>
<td>human</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Inulin (oligofructose)</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
<td>35,88</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td></td>
<td></td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Okara</td>
<td>rodent</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
<td></td>
<td>15,51</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>rodent</td>
<td></td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>PolyGlycopleX (PGX)</td>
<td>rodent</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Psyllium</td>
<td>rodent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>55,87</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>1,8,52</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>rodent</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

"+" indicates dietary fiber affects this property positively or negatively. "-" indicates dietary fiber did not have an effect on this property. TG= triglycerides
Table 3. Summary of the effects fiber on weight and biomarkers related to Type II diabetes

<table>
<thead>
<tr>
<th>Fiber</th>
<th>Species</th>
<th>Weight</th>
<th>Plasma Glucose</th>
<th>GTT</th>
<th>Plasma Insulin</th>
<th>ITT</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Cyclodextrin</td>
<td>rodent</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>β-Cyclodextrin</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>49</td>
</tr>
<tr>
<td>β-glucan or barley</td>
<td>rodent</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Blueberry peel extract</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59</td>
</tr>
<tr>
<td>Cassia tora Linn.</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>53</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose</td>
<td>rodent</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16,92</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>rodent</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Galactomannan (guar)</td>
<td>human</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>Inulin (oligofructose)</td>
<td>rodent</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Okara</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15,51</td>
</tr>
<tr>
<td>Pectin</td>
<td>rodent</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41,67</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>PolyGlycopleX (PGX)</td>
<td>rodent</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Psyllium</td>
<td>rodent</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>12, 55, 90</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1, 7, 52</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>rodent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
</tbody>
</table>

"+" indicates dietary fiber affects this property positively or negatively. "-" indicates dietary fiber did not have an effect on this property. GTT= glucose tolerance test; ITT= insulin tolerance test.
2.8 References


CHAPTER 3

The modulation of bio-behaviors by a short-course high fat diet in juvenile mice

3.1 Introduction

In 1997, the World Health Organization (WHO) declared the global increase in obesity one of pandemic proportions (1). The prevalence of juvenile obesity alone has more than tripled in the last 30 years (2). The Center for Disease Control (CDC) defines obesity in adolescents as at or over the 95th percentile of sex-specific body mass index (BMI) for age growth charts and overweight as over the 85th and under the 95th percentile (3). An NHANES study found 31.5% of children overweight and 16.0% of children obese in 2000-2001. In addition, 11.4% of infants and 10% of 2 to 5 year olds were found to be overweight (4). Especially concerning is the accompanying rise in previously “adult-only” co-morbidities such as hypertension, hyperglycemia, insulin resistance, Type II diabetes, cardiovascular disease, and stroke (2, 4, 5). Furthermore, increasing epidemiological evidence suggests an association between obesity and reduced cognitive function and psychiatric disorders such as attention deficit hyperactivity disorder (ADHD) and dementia in both children and adults (6-12). Several epidemiological studies have found a negative association between obesity or overweight and decreased performance on tests of executive function (impulsivity, decision making, and problem solving) and attention (13). In addition, the prevalence of ADHD is greater in obese and overweight cohorts compared to controls (14, 15). Altered dopaminergic function has been implicated in both disorders, alluding to the possibility of a shared mechanism (16).

In humans, a critical stage in brain development, the brain growth spurt, begins in the third trimester of pregnancy and continues to approximately age 3 (17, 18). The brain growth spurt period is associated with a rapid increase in brain weight, proliferation of astroglial or oligodendroglial cells, neuronal axonal elongation and dendritic arborization and synaptogenesis (19). The developing brain is especially vulnerable to insult by environmental factors such as diet (17, 20, 21). The juvenile mouse is an appropriate model for studying the effects of a high fat diet on the developing brain as a similar brain growth spurt period occurs in mice within the first few weeks following birth. In addition, a host of behavioral characteristics can be measured in the mouse such as spontaneous locomotion, anxious- and depressive-like behavior, and cognition. Several studies have reported that a high fat diet impairs learning.
acquisition and both non-spatial and spatial memory in adult rodents (22-26). While a high fat diet seems to have an effect on learning and memory in older rodents fed a high diet fat for an extended period of time, little exploration has been made into the immediate effect of a high fat diet on the juvenile mouse. In one of the few studies investigating short-term high fat diet feeding effects, cognition was negatively affected in 2 mo old rats following 9 d of feeding a 55% fat diet (27). Here we placed 3 wk old mice on a 60% fat (HFD) or a 10% fat (LFD) diet and performed behavioral tests beginning after 1 wk of feeding to determine the immediate effects of a HFD on bio-behaviors, learning, and neuro-immune function in juvenile mice. We further used knockout mouse models and pharmaceutical intervention to explore mechanism. To our knowledge, this is the first study that has investigated the immediate effect of a HFD on bio-behavior in juvenile mice.

3.2 Methods

Animals- C57BL/6J male mice (3 wk old) were purchased from Jackson Laboratories (Bar Harbor, ME). IL1R1 -/-, IDO -/-, and TLR4 -/- male mice were bred in house and weaned at 3 wk old. Mice were group housed (4-8 per cage) in large standard shoebox cages (length 28 cm; width 17 cm; height 12.5 cm) and allowed free access to food and water. Housing temperature (72°F) and humidity (45-55%) were controlled, as was a 12/12 h reversed dark-light cycle (2200-1000 h). Video recording of animal behavior was performed under red light using a night shot capable video camera (Sony HDR-XR500V) except in the zero maze test where mice were recorded in white light to induce anxiety. Animal use was conducted in accordance with Institutional Animal Care and Use Committee (IACUC) approved protocols at the University of Illinois, Urbana.

Diets, weight, and fasting blood glucose - Mice were fed open source uniform-base diets containing either 10% calories from fat (LFD) (D12450B, Research Diets, New Brunswick, NJ) or 60% calories from fat (HFD) (D12492, Research Diets, New Brunswick, NJ). Both diets contained 20% kcal from protein (casein). The LFD contained 70% kcal from carbohydrates (corn starch) and 10% calories from fat (soybean oil and lard). The HFD contained 20% kcal from carbohydrates (corn starch) and 60% calories from fat (soybean oil and lard). Mice had free access to food and water for study duration (1 to 3 wk). Mouse weight was recorded weekly.
using an Adventurer Pro digital scale (Ohaus, Parsippany, NJ). Mice were fasted for 12 hrs prior to tail blood collection. Fasted mice were provided water *ad libitum*. Mouse tail blood glucose was recorded weekly using a FreeStyle Freedom blood glucose monitor (Abbott, Abbott Park, IL) after the tail was cleaned with 70% ethanol and lanced with a sterile No. 10 surgical scalpel blade (Feather, Osaka, Japan).

**Quantitative PCR (qPCR)** - Mice were sacrificed after 1 or 3 wk of feeding by CO₂ asphyxiation. Brains were perfused with 1% phosphate buffered saline (PBS) to eliminate contaminating blood, and brain sections (hippocampus, cortex, and hypothalamus) were collected in 1 mL Trizol (Invitrogen, Carlsbad, CA) and stored on ice until homogenization with the Tissuelyser II (Qiagen, Hilden, Germany). Homogenate then was stored at -80 °C until RNA extraction was performed. RNA isolation from brain sections was performed using a lipid tissue mRNA extraction kit (Qiagen, Hilden, Germany). RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (PN 4368813) (Applied Biosystems, Carlsbad, CA). The TaqMan Gene Expression primers used were: F4/80 (Mm00802529_m1), IL-1α (Mm99999060_m1), IL-1β (Mm99999061_mH), IL1R1 (Mm00434237_m1), IL1R2 (Mm00439622_m1), IL1RA (Mm01337566_m1), IL-6 (Mm01210733_m1), CD11b (Mm00434455_m1), tumor necrosis factor- α (TNF-α) (Mm00443258_m1), CD45 (Mm00448490_m1), tyrosine hydroxylase (TH) (Mm00447554_m1), cyclic AMP responsive element binding protein-1 (CREB1) (Mm00501607_m1), brain derived neurotrophic factor (BDNF) (MM01334042_m1), ARG/ARC (Mm01190441_g1), and nerve growth factor (NGF) (Mm00443039_m1). qPCR was performed on a 7900 HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA) using TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA). To compare gene expression, a parallel amplification of endogenous Rps3 (Mm00656272_m1) was performed. Reactions with no reverse transcription and no template were included as negative controls. Relative quantitative evaluation of target gene to rps3 was performed by comparing ∆ΔCₜₚ, where Cₜ is the threshold concentration.

**Behavioral Tests:**

**Spontaneous Alternation (Y-maze)** - Spontaneous alternation is used to access locomotion and hippocampal-dependent spatial memory. Alternation utilized a symmetrical 3-arm, clear
Plexiglas Y-maze (arms = 40 cm in length x 9 cm in width x 16 cm wall in height with an arm angle of 120°). Maze bottom was blue and side walls were decorated with either black triangles, black circles, or black diagonal lines. Mice were single housed in small standard shoe box cages overnight prior to testing. To initiate testing, the subject mouse was randomly placed in one of the 3 maze arms. Movement was recorded for 5 min and evaluated from the video record. Results are presented as total arm entries, perfect alternations, and imperfect alternations. Perfect alternations were defined as exploration of all three arms sequentially given 3 opportunities independent of a right or left arm choice at initiation. Imperfect alternations were defined as entry into all three arms in 4 opportunities. Score was calculated as the number of perfect or imperfect alternations divided by total opportunities. To have entered an arm the mouse was required to have all 4 legs in the arm.

**Novel Object Recognition Test** - The novel object recognition test (NOR) is a test of non-spatial cortex-dependent memory that exploits an animal’s propensity to explore a novel object more than a familiar object. Mice were single housed in small standard shoe box cages with 2 identical objects (familiar objects) per cage overnight prior to testing. For the acclimation period, mouse was placed in a large standard cage with light bedding (novel arena) containing the familiar objects. Objects were placed 10 cm apart at the short-side wall end 5 cm from the short side wall and 6.5 cm from the long-side wall. Mice were recorded for 5 min and then returned to home cages for 1 h. For testing, mice were placed in the novel arena containing one familiar object (cleaned with 70% alcohol prior to reuse) and one novel object. Objects used included a glass stone, a 6-sided plastic die, or a shaped constructed from small building blocks. Exploratory behavior was video-recorded for 5 min and evaluated from video record. Object exploration was defined as the mouse touching the object with nose or paws. Sitting on object was not considered exploration. Data are presented as percent exploration of the novel object. Percent investigation was calculated by dividing the time spent examining the novel object by the total time spent investigating both objects multiplied by 100.

**Locomotor Activity** - The locomotor activity test is used to assess spontaneous movement. Mice were single housed overnight prior to testing. For testing, nestlet was removed from home cage and cages were placed on the floor. Mice were examined in their home cage by video recording
for 5 min. Movement was quantified using EthoVision XT 7 (Noldus Information Technology, Leesburg, VA).

Wheel running activity- Voluntary wheel running behavior is considered a self-rewarding activity as well as a measure of activity level. Mice were single housed in standard cages containing a stainless steel running wheel (Mini-Mitter, Bend, OR) 2 h prior to start of dark cycle. Wheel turn data were collected via a magnetic reed switch that sent the information to a computer with VitalView Software (Mini-Mitter, Bend, OR). Data were collected for 1 h intervals and summed for 24 h wheel turns. Data are presented as total wheel turns for first 24 h period after either 1 wk or 3 wk of diet feeding.

Burrowing- Burrowing is an innate behavior in mice used to test anxiety and reward seeking behavior. Burrows were constructed of 20.3 cm long ASTM D2665 polyvinyl chloride (PVC) pipe fitted at one end with an ASTM D2665 PVC pipe cap (closed end). The open end was raised 1.3 cm on twin steel legs. To acclimatize mice to the burrow, mice were single housed in large standard cages with burrow present overnight prior to testing. Testing was initiated 2 h prior to the dark cycle by adding 100 g of food pellets to the burrow. Prior to replacing the burrow filled with food (burrow + food) back into the cage, the burrow + food was weighed. The burrow then was placed horizontal to the cage long-side wall with the closed end against the cage short-side wall. Water was provided ad libitum, but food was only available from the burrow. Mice were allowed to dig and or eat the food out of the burrow for 30 min. Burrows were then removed and weighed. Amount burrowed was calculated by subtracting the burrow + food weight before and after the 30 min period of burrowing.

Swim test- The forced swim test is used to measure depressive-like behavior in mice by assessing mobility. Decreased activity in the forced swim test is indicative of increased depressive-like behavior. Mice were single housed in standard cages overnight prior to testing. Testing was initiated by individually transferring mice from their home cage to a clean white cylindrical PVC container (diameter 16 cm; height 31 cm) containing 15 cm of water maintained at 25 ± 1°C. Total swim duration was 6 min and immobility was evaluated from the video record
encompassing the final 5 min of the swim. After testing, mice were patted dry with paper towels and dried under a heat lab and then returned to their home cage.

_Elevated Zero Maze_- The elevated zero maze is used to measure anxiety-like behavior in mice by assessing the amount of time spent in an open area of an elevated platform. The zero maze utilized a circular platform (6 cm width with a 40 cm inner diameter) elevated 72 cm from the ground. The maze is equally divided into 4 quadrants, 2 of which are enclosed by walls (14 cm high) and two quadrants that are open and bordered by a lip 0.3 cm high. To initiate testing, the mouse was placed in closed section of zero maze and video recorded in white light for 5 min. Video record was evaluated for percent time spent in open areas and number of head dips. Percent time was calculated as time spent in open area divided by total time multiplied by 100. A head dip was considered as mouse’s head extending beyond the maze lip in the open area.

_Pharmaceutical Intervention_- To correct non-spatial memory deficit, stimulants or antidepressants were administered prior to the acclimation phase of novel object testing. Mice were injected intraperitoneally with caffeine (25 mg/kg) (Bedford Labs, Bedford, OH), reboxetine (1 mg/kg) (Sigma Aldrich, St. Louis, MO), desipramine (1 mg/kg) (Sigma Aldrich, St. Louis, MO), or methylphenidate (2.5 mg/kg) (Medisca, Quebec, Canada) after overnight object acclimation, 45 min prior to novel object acclimation phase.

_Statistical analysis_- Data analysis was conducted using Sigma Plot 11.2 (Systat Software, Chicago, IL). Weight and behavioral data were subjected to 1-way analysis of variance (ANOVA) to determine the main effects of diet (LFD or HFD) followed by Tukey adjustment. When normality failed (P<0.05), the Kruskal-Wallis 1-way analysis of variance was performed followed by a Tukey adjustment when group size was equal and a Dunn’s adjustment in cases where group size was not equal. qPCR data were analyzed using unpaired t-tests to determine differences between diet groups. Statistical significance was assumed at P<0.05 and all data are presented as means ± SEM.
3.3 Results

HFD induces weight gain and elevation of fasting blood glucose
HFD-fed mice gained significantly more weight than LFD-fed mice after 21 d of feeding. (22.26g±0.73 vs. 19.83g±0.58) and had significantly elevated fasting blood glucose concentrations after 14 d of feeding (148.38mg/dL±7.90 vs. 109.69mg/dL±11.62). There was no difference in weight between diet groups after 7 (17.18g±0.61 vs. 18.64g±0.83) or 14 (18.23g±0.61 vs. 20.15g±0.82) d of feeding, and no difference in fasting blood glucose levels after 7 d of feeding (105.63mg/dL±9.16 vs. 108.94mg/dL±5.42) (Figure 1A-B).

HFD increases wheel running, but not spontaneous locomotor activity
HFD-fed mice had increased wheel running activity (greater wheel turns/d) compared to mice fed a LFD after 7 d of feeding (2914.5±492.51 vs. 1242.75±283.16), while there was no significant difference in wheel running activity between diet groups after 21 d of feeding (4122.00±938.28 vs. 3679.38±1161.80) (Figure 2A). There was no difference in spontaneous locomotor activity (total distance traveled or velocity) between the HFD and LFD groups after 7 or 21 d of feeding (Figure 2B,C).

HFD increases anxieta- but not depressive-like behavior
HFD-fed mice burrowed a significantly greater weight of food pellets than LFD-fed mice after 7 d of feeding (45.61±9.04 vs. 14.91±7.15) and 21 d of feeding (87.19±6.35 vs. 46.83±11.12) (Figure 3A). In the forced swim test, HFD-fed mice were significantly less immobile after 21 d of feeding (243.113±19.85 vs. 212.82±14.8) while there was no difference in immobility between diet groups after 1 wk of feeding (239.93±28.43 vs. 271.81±23.24) (Figure 3B). HFD-fed mice spent less time in the open area of the zero maze after 7 d of feeding (52.46±6.48 vs. 36.91±2.90) while there was no difference in time spent in open area between diet groups after 21 d of feeding (49.53±3.29 vs. 42.89±5.26) (Figure 3C) or in number of total head dips after 7 or 21 d of feeding (data not shown).

HFD impacts non-spatial and spatial memory
In the novel object recognition test, a test of non-spatial memory, mice fed a HFD explored a novel object less than LFD-fed mice after 7 d of feeding (50.80±4.21 vs. 72.00±1.75) and 21 d of
feeding (54.76±3.55 vs. 81.5±22.09) (Figure 4A). In the Y-maze, a test of spatial memory, HFD-fed mice had decreased perfect alternations compared to LFD-fed mice after 7 d of feeding (0.66±0.02 vs. 0.58±0.03), while there was no difference after 21 d of feeding (0.66±0.03 vs. 0.61±0.03). There also was no difference in total arm entries (7 d: 30.75±1.55 vs. 31.38±1.60; 21 d: 126.06±0.03 vs. 26.44±0.99) or imperfect (7 d: 0.82±0.01 vs. 0.80±0.02; 21 d: 0.85±0.03 vs. 0.83±0.02) alternations between HFD- and LFD-fed mice at 7 or 21 d on diet (data not shown).

**LFD feeding restores novel object recognition in HFD fed mice**

Novel object recognition was restored in mice fed a HFD for 7 d followed by LFD feeding for 7 d. After 7 d of feeding, HFD- and LFD-fed mice explored a novel object differentially (67.21±2.82 vs. 51.50±3.56). HFD mice then were switched to a LFD. After 7 d of additional feeding, mice were retested using a different novel object. Both diet groups explored a novel object equivocally at retest, at greater than 70% exploration (78.78±2.82 vs. 70.05±3.26) (Figure 5).

**HFD does not affect NOR in IL1R1 /- or IDO /- mice**

IL1R1 /- mice explored a novel object equivocally (approximately 50% of total exploration) regardless of diet or time on diet (7 d: 53.16±3.28 vs. 5.19±3.90; 21 d: 50.86±3.98 vs. 56.33±4.26) (Figure 6A). HFD-fed IDO /- mice explored a novel object significantly less than LFD-fed IDO /- mice (65.37±2.33 vs. 51.84±3.89) (Figure 6B).

**HFD impacts expression of brain cytokines and growth factors related to inflammation, memory, and learning**

Figure 7 (A-E) shows HFD-fed mice when compared to LFD-fed mice have differential mRNA expression of IL-6, BDNF, NFG, IL1R2, IL1R2, and IL1RA that is dependent on brain region and time. IL1-α, IL1-β, F4/80, CD45, TNF-α, CD11b, ARG/ARC, TH, and CREB1 mRNA were expressed at similar levels irrespective of diet, time, or brain regions (data not shown).

**Methylphenidate administration corrects non-spatial memory deficit in HFD-fed mice, while anti-depressants and caffeine do not**

Administration of reboxetine and desipramine resulted in equivocal exploration of a novel object between drug injected LFD and HFD mice of approximately 50-55% of total exploration, indicating an inability to discriminate between novel and familiar objects (reboxetine:...
56.48±5.39 vs. 51.34±5.39; desipramine: 56.95±5.79 vs. 47.69±2.22) (Figure 6A-B).
Administration of methylphenidate resulted in equivocal exploration of a novel object between
drug-injected LFD- and HFD-fed mice (60.03±1.86 vs. 66.11±5.32), equal to novel object
exploration of vehicle-injected LFD-fed animals (Figure 6C). Administration of caffeine
resulted in differential exploration of the novel object by LFD- and HFD-fed drug-injected mice,
although the difference did not reach significance (60.73±3.88 vs. 39.97±4.53).

3.4 Discussion
Epidemiological evidence indicates there is an association between obesity, depression,
anxiety, and cognitive decline in both adults and adolescents (7, 28-30). As the prevalence of
obesity and associated co-morbidities rises within the juvenile population, an understanding of the
mechanism by which a HFD affects these characteristics in juveniles becomes critical. Here, we
demonstrate that a HFD increases reward-seeking and anxious-like behavior and induces memory
deficit after 7 d of feeding post-weaning independent of significant change in body weight or
elevation in fasting blood glucose. Interestingly, increased anxiety, reward-seeking, and cognitive
deficit also are seen in obese cohorts and in children and adults with attention deficit disorder
(ADHD) (13, 14).

After 7 d of HFD feeding, juvenile mice burrowed 66% more compared to mice fed a
LFD and continued to have increased burrowing (40% greater) after 21 d of feeding compared to
LFD-fed mice. Traditionally, changes in burrowing activity have been equated to anxiety-like
behavior, as burrowing is utilized in nature to hide from predators and conceal food (31). This
may not be the case in a laboratory setting, as mice will burrow or kick out substrate from a
container in a fashion similar to the “spring cleaning” behavior seen in wild field mice, even if an
empty container is present (32). Thus, burrowing behavior may be interpreted as a reward
behavior, a behavior that mice find pleasurable. In fact, Sherwin et al. demonstrated mice are
motivated to burrow and can be taught to press a lever to access burrowing material, even when
no immediate need to burrow is present (33). Further behavioral testing revealed both increased
anxiety and increased reward seeking behavior in HFD-fed mice after 7 d of feeding.
Voluntary wheel running activity also can be viewed as a reward behavior (34). Here, HFD-fed mice had 48% greater wheel turns in the 24 h period following 7 d of diet feeding compared to LFD-fed mice. This increase in activity seen in burrowing and wheel running cannot be attributed to an increase in general locomotor activity resulting from HFD consumption as there was no difference between diet groups in terms of spontaneous locomotor activity or total arm entries in the Y-maze. We previously demonstrated a 35% decrease in spontaneous locomotor activity in adult HFD-fed mice that were fed for 12 wk compared to LFD-fed mice (35). This could have been due to a large significant weight difference and differences in fasting blood glucose between the two diet groups. Here, after 7 d of feeding, there was no significant difference between HFD- and LFD- fed mice in terms of weight or fasting blood glucose. While spontaneous movement may be decreased in adult HFD-fed mice, several studies have demonstrated an initial increase in wheel running activity in adult HFD-fed mice compared to a control (34, 36). In contrast, Ma et al. did not find a difference in wheel running activity between HFD-fed and control-fed mice, but mice were 7 wk old when placed on a HFD compared to previous studies that placed mice on study diets within 0-2 wk of weaning (37). This suggests that a HFD may have an effect on brain development, as changes in reward-seeking activity are only seen when mice are placed on a HFD at weaning.

In addition to an increase in reward-seeking and anxieta-like behavior, we found that a HFD has an immediate, detrimental effect on non-spatial and spatial memory. Here we used the novel object recognition test (NOR) to assess non-spatial memory as it does not use an external reward and does not induce additional anxiety with water or food restriction. The NOR tests a mouse’s ability to discriminate between a familiar and novel object. HFD-fed mice explored a novel object 29% less often than LFD-fed mice after 7 d of feeding and 32% less after 21 d of feeding. Several studies have demonstrated impaired NOR in adult mice fed a 45% fat diet compared to a control diet (23, 24). In contrast, we previously found no difference in NOR in adult mice fed a 60% fat diet compared to mice fed a 10% fat diet (35). This may be due to differences in diet composition or experimental design. Acclimation to familiar objects in this study was longer than in previous studies. In this study, mice were acclimated to the familiar object overnight and we used larger, more complex objects. HFD feeding may affect spatial and non-spatial memory differentially. In addition, non-spatial memory deficit in NOR was reversed
by removal of the HFD. When HFD animals were switched to a LFD, memory deficit was corrected within 7 d, indicating that the effect of diet on the brain was transient, rather than a result of permanent damage. In this study, HFD-fed mice also demonstrated a deficit in spatial memory measured by the spontaneous alternation paradigm, a measure of hippocampal-based learning. Interestingly, Kanoski et al. found after 72 h of HFD feeding rats showed an immediate deficit in spatial memory, but not non-spatial memory. Non-spatial memory remained intact until 30 d of HFD feeding (38). The radial arm maze, a hippocampal-based learning task, was used to test both spatial and non-spatial memory. NOR is a cortex-based test, suggesting a HFD diet has an immediate effect on the cortex-based learning as well as hippocampal-based learning.

We also did not find differences in depressive-like behavior as measured by the forced swim test. Interestingly, HFD-fed mice were less immobile in the forced swim test compared to LFD-fed mice after 21 d of feeding. Several studies have demonstrated decreased depression and anxiety in adult HFD-fed animals indicating long-term HFD feeding may be protective for stress-induced anxiety and depression (35, 39).

The IL-1 cytokine super family (IL-1α, IL-1β, IL1R1, IL1R2, and IL1RA) is associated with learning and memory (40-42). IL-1 is required for memory consolidation and learning, especially hippocampal-based memory (43). In fact, IL-1β expression is induced following spontaneous alternation testing (44). IL-1 has not been found to play a role in non-spatial memory (45). In contrast, LFD- and HFD-fed IL1R1 -/- mice explored a novel object equivocally, approximately 50% of total exploration time after 7 and 21 d of feeding, indicating novel object recognition is impaired in IL1R1 -/- mice regardless of diet. This suggests IL-1 plays a role in non-spatial memory and learning and is critical in NOR. A previous study that did not find a non-spatial memory deficit used a visually guided water maze test (45). The NOR may be a more sensitive measure as it does not have a stress component. However, no difference in IL-1 cytokines was found in the brain after 1 wk of feeding. Slight differences in IL1R2 and IL1RA were seen after 21 d of feeding. IL1R2 mRNA was decreased in the hypothalamus of animals fed a HFD for 21 d and IL1RA mRNA was increased in the cortex of mice fed a HFD for 21 d. IL1RA competes with IL-1α and IL-1β for binding to the IL-1 receptors in the brain (40, 41). Blocking of IL-1 receptors by IL1RA in the hippocampus has been shown to enhance memory
retention in avoidance tasks, but does not have an effect in habituation to novel environments (40). We previously demonstrated an increase in IL1RA in the serum of adult HFD-fed mice (46). IL1RA also has been shown to be elevated in the serum of obese humans, but the exact role of IL1RA in metabolism is unknown (47). Our study suggested elevated IL1RA in obesity may be protective against hypoxia (46). The expression of all other IL1 family cytokines was equivocal between diets regardless of time or brain region indicating IL-1 does not play a role in HFD-induced memory deficit seen in this study.

Obesity is associated with a pro-inflammatory state (48-50), and elevated expression of TNF-α and IL-6 in the brain has been associated with cognitive decline (50). However, no differences in expression of TNF-α or macrophage activation markers (CD45, CD11b, and F4/80) were found in hippocampus, hypothalamus, and cortex of the brain after 7 and 21 d of diet feeding. There was a 13% decrease in expression of IL-6 in the hypothalamus of mice fed a HFD for 7 d compared to LFD-fed mice and a 15% decrease in expression of IL-6 in the hippocampus and a 23% decrease of IL-6 in the cortex of HFD-fed mice after 21 d of feeding. In contrast to our findings, elevation in IL-6 in the brain has been associated with cognitive decline and brain disorders (51). Furthermore, IL-6 deficient animals have increased cognition compared to age-matched wild type mice (52). Studies that have examined the relationship between obesity, IL-6, and cognition have done so in an elderly human population or in adult mice fed a HFD for an extended period of time (50). Adipocytes are the main source of pro-inflammatory cytokines in the obese state (48, 49). Here, mice are 4-6 wk old and exposed to a HFD for only 7 or 21 d, with the HFD-fed group becoming significantly heavier than the LFD-fed group after 12 d of feeding, whereas bio-behaviors are affected after 7 d of HFD feeding. Reaching the pro-inflammatory state previously seen in mouse models of obesity may take longer to develop, suggesting an alternate pathway for cognitive impairment in a juvenile model.

Alternations in the kynurenine pathway also have been implicated in neuro-cognitive deficit, depression, ADHD, and inflammation (51, 53). Interestingly, children with ADHD have been found to have elevated levels of serum tryptophan compared to controls, implicating alteration of the kynurenine pathway (51). Additionally, kynurenic acid, a product of the kynurenine pathway, can be neuroprotective (54). Here mice deficient in indoleamine 2,3
dioxgenase (IDO-/-) did not differ in performance in the NOR test from control mice, indicating IDO is not involved in HFD-induced memory deficit.

We also examined established markers for learning and memory: tyrosine hydroxylase (TH), CAMP responsive element binding protein 1 (CREB-1), brain-derived neurotropic factor (BDNF), and nerve growth factor (NGF). While there was no difference in expression of CREB-1 and TH, expression of BDNF in the cortex was decreased 17% in mice that were fed a HFD for 7 d. BDNF has been associated with synaptic plasticity and memory processes including cortex-mediated object recognition (55-57). A high fat diet has been shown to decrease BDNF in the hippocampus and, furthermore, decreases in BDNF in the hippocampus also have been associated with impairment in working memory and decreased spontaneous alternation performance (55, 57). BDNF is closely related to the dopamnergic pathway and dopamnergic functions (58, 59). Dopamine agonists have been shown to regulate expression of BDNF, indicating dopamine is a regulator of BDNF in the prefrontal cortex (55). Dopamine levels in the cortex are thought to be related to cognition, while dopamine levels in the striatum are thought to be related to motivation (16). In addition to being part of the mesolimbic dopamine reward system, BDNF regulates food intake and body weight (60, 61).

Methylphenidate (MPH), a psychostimulant, is a dopamine transport inhibitor commonly used to treat attention deficit hyperactivity disorder (ADHD). MPH increases the synaptic concentration of dopamine and norepinephrine by blocking monoamine transporters in the striatum, thus inhibiting reuptake (62). Here, administration of MPH corrected HFD-induced non-spatial memory deficit in the NOR test. Psychostimulants also may increase norepinephrine and serotonin. Norepinephrine re-uptake inhibitors, desipramine, which is also a selective serotonin re-uptake inhibitor, and reboxitene, which does not inhibit serotonin re-uptake did not correct HFD-induced memory deficit, indicating in this model that alterations in dopamine and not norepinephrine or serotonin have an impact on memory.

ADHD and obesity share similar clinical manifestations such as impaired executive function and increased impulsivity, and similar alterations of growth factors and the dopaminergic pathway suggesting a common mechanism. This study supports this as HFD feeding immediately
increased ADHD-like bio-behaviors and alterations of growth factors related to the dopaminergic pathway in the cortex. The dopaminergic system in the frontal cortex is especially sensitive to changes in essential fatty acids (63). Further research is needed to elucidate the mechanism by which a HFD alters the dopaminergic pathway leading to ADHD-like symptoms and obesity.
3.5 Figures

Figure 1: *HFD results in weight gain and elevated fasting blood glucose after 7 d of feeding.* Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. Weight (A) and fasting blood glucose (B) were measured weekly. White bar indicates LFD and black bar indicates HFD. *P≤0.01, **P ≤0.001; n=8
Figure 2: HFD increases wheel running but not spontaneous locomotor activity.

Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. To measure activity, (A) voluntary wheel running activity (n=16) and (B,C) spontaneous locomotion (n=8) were measured after 7 or 21 d of feeding. White bar indicates LFD and black bar indicates HFD. *P≤0.001
Figure 3: HFD increases reward seeking behavior and anxietal-like behavior, but not depressive-like behavior.

Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. To measure reward, anxietal, and depressive-like behavior, mice were subjected to the (A) burrowing test (*P≤0.01, n=8; **P ≤ 0.03, n=11), (B) forced swim test (*P ≤ 0.02, n=16), or (C) zero maze (*P≤0.05, n=15-16) after 7 or 21 d on diet. White bar indicates LFD and black bar indicates HFD.
**Figure 4**: *HFD decreases novel object recognition and spontaneous alternation.*

Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. To measure non-spatial and spatial memory, mice were subjected to either the (A) novel object recognition test (*P≤0.001, n=16) or (B) Y-maze test for spontaneous alternation (*P≤0.02, n=16) after 7 or 21 d of feeding. White bar indicates LFD and black bar indicates HFD.
Figure 5: Novel object recognition was restored in HFD-fed mice by feeding a LFD.

Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. To determine whether novel object recognition could be restored by a LFD, mice were fed a LFD or HFD for 7 d, subjected to novel object recognition test, then HFD-fed mice were switched to LFD (HF->LFD) for 7 d. Novel object recognition was re-tested after 7 d of additional feeding. (*P ≤ 0.001, n=16). White bar indicates LFD, black bar indicates HFD and grey bar indicates HF->LFD.
**Figure 6:** *IL1R1−/−* mice explored a novel object equivocally, while *IDO−/−* mice explore a novel object differentially.

*IL1R1−/−* and *IDO−/−* mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. (A) In *IL1R1−/−* mice, novel object recognition was tested after 7 or 21 d of feeding (n=10-12). White bar indicates LFD fed knockout mice and black bar indicates HFD fed knockout mice. (B) *IDO−/−* (IDO) or wild type (WT) mice were placed on a low fat (LFD) or high fat (HFD) diet at weaning. Mice were subjected to the novel object recognition test after 1 wk of feeding. White bar indicates LFD-fed wild type mice, black bar indicates HFD-fed wild type mice and light grey bar indicates LFD-fed knockout mice and dark grey bar indicates HFD-fed knockout mice. Different letters indicate significant differences among groups (P<0.05, n=12).
Figure 7: HFD feeding impacts cytokine and growth factor expression in the hippocampus, hypothalamus, and cortex.

Mice were placed on a low fat diet (LFD) or high fat diet (HFD) at weaning. Brain sections were harvested at 7 or 21 d of feeding and cytokine and growth factor mRNA expression were measured using qPCR. (A) IL-6 (*P≤0.03, #P ≤ 0.02, n=8) (B) BDNF (*P ≤ 0.006, n=8) (C) NGF
(*P ≤ 0.04, n=8) (D) IL-1R2 (*P≤0.02, n=8) (E) IL-1RA (*P ≤ 0.05, n=8) White bar indicates LFD and black bar indicates HFD. LFD at 7 days is set to 1 for comparison. BDNF= brain-derived neurotrophic factor; NGF= nerve growth factor; Hi=hippocampus; Hy=hypothalamus; C=cortex
Mice were fed a low fat diet (LFD) or high fat diet (HFD) for 1 wk. The novel object test was performed after the administration of the following IP: (A) reboxitine (n=8), (B) despiramine (n=8), (C) methylphenidate (n=8), (D) caffeine (n=4). White bar indicates vehicle-injected LFD-fed mice, black bar indicates vehicle-injected HFD-fed mice, light grey bar indicates drug-injected LFD-fed mice and dark grey bar indicates drug-injected HFD-fed mice. Differences in letters indicate significant difference between groups, P<0.05.
3.6 References


cognitive function in the presence of obesity and hypertension: the Framingham heart study. *Int.

obesity and cognitive function across the lifespan: implications for novel approaches to

dysfunction. *Nat. Protoc.* 1: 118-121.

32. Deacon, R. M. 2009. Burrowing: a sensitive behavioural assay, tested in five species of


Freund. 2011. Fasting induces an anti-inflammatory effect on the neuroimmune system which a
high-fat diet prevents. *Obesity* 19: 1586-1594.

Turek, and J. Bass. 2007. High-fat diet disrupts behavioral and molecular circadian rhythms in

Peng, U. M. Kujala, P. Rahkila, and H. Suominen. 2010. Effects of diet-induced obesity and
voluntary wheel running on bone properties in young male C57BL/6J mice. *Calcif. Tissue Int.*
86: 411-419.


CHAPTER 4
Modulation of outcomes pertaining to diabesity by dietary fiber

4.1 Introduction

Dietary fiber (DF) has been heralded as a solution to the diabesity crisis, but research pertaining to obesity and diabetes is inconsistent (1-8). Furthermore, the mechanism by which DF reduces the risk of these conditions remains to be elucidated. Epidemiological (9-12) and animal studies (2, 3, 13-16) lend evidence toward an inverse relationship between DF intake and weight, while data from human intervention trials has been less convincing (7, 17-20). Additionally, statistically significant weight loss resulting from DF intake in human trials is not always clinically relevant (18). DFs appear to be most effective in weight reduction in individuals who are obese or overweight at baseline and when used in combination with a calorie-restricted diet (11, 17). The relationship between DF intake and diabetes is also unclear. Animal (2, 21) and human (6, 7) research indicates a protective effect of soluble DF against hyperglycemia, but this relationship is not reflected in population-based studies (22).

The effect of DF on weight and blood metabolites may be dependent on DF dose, type, and the intrinsic properties of the DF. The characterization of DF and the role of DF in metabolism are extensively reviewed in Chapter 2. Briefly, DF is classified by solubility, degree of microbial fermentation, and viscosity (19). Through these physical properties, DF confers health benefits by reducing macro-nutrient absorption through increased viscosity of luminal contents, delayed gastric emptying, and by increased fecal bulking (1, 23, 24). As an example, pectin is a soluble, viscous, and highly fermentable DF that is completely digested in the colon (25). Cellulose, on the other hand, is an insoluble, non-fermentable DF that is virtually indigestible. Additionally, cellulose binds water in the large intestine, increasing fecal bulk (25). Other fibers such as resistant starch and oligosaccharides fall somewhere in the middle on the solubility and fermentation scales. Fructooligosaccharides are indigestible in the upper gastrointestinal tract, but are rapidly fermented by microbes in the proximal colon. Resistant starches also are indigestible and fermentable, but are more slowly degraded and undergo fermentation in the distal colon (26, 27).

Here, we re-visit pectin, a main focus of fiber research in the 1970’s, as well as examine resistant starch and fructooligosaccharides, two relative newcomers to fiber research, to re-
explore the role of DF in the prevention of weight gain and hyperglycemia. We compare the ability of pectin, resistant starch, fructooligosaccharides, and cellulose to slow weight gain when added to a high-fat diet (HFD). In addition, we explore pectin’s role in the prevention of diabesity by examining the capability of pectin to bind lipids and alter intestinal morphology.

4.2 Methods
Animals- C57BL/6J male mice (3 wk old) were purchased from Jackson Laboratories (Bar Harbor, ME). MYD88−/−, TLR2−/−, TLR4−/−, IL1R1−/−, and IL4−/− male mice were bred in house and weaned at 3-4 wk. C57Bl/6J, IL4−/−, and IL1R1−/− mice were group housed (8 per cage) in large standard shoebox cages (length 28 cm; width 17 cm; height 12.5 cm). MYD88−/−, TLR2−/−, and TLR4−/− mice were group-housed in small standard shoebox cages housed in a ventilated cage system. Mice were allowed free access to food and water. Housing temperature (72°F) and humidity (45-55%) were controlled, as was a 12/12 h reversed dark-light cycle (2200-1000 h). Animal use was conducted in accordance with Institutional Animal Care and Use Committee (IACUC)-approved protocols at the University of Illinois.

Diets - Mice were fed open source uniform-base diets containing either 10% calories from fat + 5% cellulose by weight (LF5C, D12450B) or 60% calories from fat + 5% cellulose by weight (HF5C, D12492) or a specially formulated diet containing 10% calories from fat + one of the following DF by weight: 5% pectin (LF5P, D10101303), 10% pectin (LFP, D06082202), 10% cellulose (LFC, D06082201), 10% Orafti P95 fructooligosaccharides (LFF, D10032401), 10% High-Maize 260 resistant starch (LFRS, D10032403), 5% pectin + 5% cellulose (LFPC, D10040401 ) or 60% calories from fat + one of the following DFs by weight: 5% pectin (HF5P, D10101304), 10% pectin (HFP, D08111803), 10% cellulose (HFC,D07102501), 10% Orafti P95 fructooligosaccharides (HFF, D10032402), 10% High-Maize 260 resistant starch (HFRS, D10032404 ), or 5% pectin + 5% cellulose (HFPC, D10040402). Diet composition is outlined in Table 1. Energy values were calculated using the following conversion factors: 4 for protein, 4 for carbohydrates, and 9 for fat. Mice were fed ad libitum for study duration. All diets were purchased from Research Diets, New Brunswick, NJ.
**Weight and blood metabolites** - Mouse weight was recorded weekly using an Adventurer Pro digital scale (Ohaus, Parsippany, NJ). To measure the effect of diet on fasting blood glucose, mice were fasted for 8 h prior to tail blood collection. Fasted mice were provided water *ad libitum*. Mouse tail blood glucose was recorded weekly using an AlphaTRAK blood glucose monitor (Abbott, Abbott Park, IL) after the tail was cleaned with 70% ethanol and lanced with a sterile No. 10 surgical scalpel blade (Feather, Osaka, Japan). To measure the effect of diet on glucose and insulin properties, the intraperitoneal glucose tolerance test (IPGTT) and the intraperitoneal insulin tolerance test (IPITT) were used. For IPGTT, mice were fasted for 4 hours prior to testing. Mice were then injected intraperitoneally with a 30% glucose solution. For IPITT, fed mice were injected intraperitoneally with 0.75U/kg insulin. For both the IPGTT and the IPITT, baseline blood glucose was tested prior to injection and at 15, 30, 45, 60, 90, 120, and 180 min after injection using methods described above.

**Food intake and fecal collection** - To measure food intake and to collect feces, 6 mice of each diet group were single housed in standard metal metabolic cages with wire bottoms after 10 wk of feeding. Food intake was recorded daily for 6 d to determine voluntary food intake. Beginning on d 7, mice were given 90% of their voluntary food intake for the next 7 d. Feces were collected and pooled per mouse daily. Mice were weighed every other day. After the 14th day, pooled fecal samples were weighed to obtain wet weight and then dried at 57°C for 2 d. Fecal samples were ground through a 0.5 mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) or a coffee grinder (Mr. Coffee, Shelton, CT). A 250 mg aliquot of each sample was heated overnight at 105°C and then weighed to determine dry matter content.

**Sample collection and histology** - Mice were sacrificed after 12 wk of feeding by CO₂ asphyxiation. Blood was collected by cardiac puncture into a 2 mL Eppendorf tube (Eppendorf, Hamburg, Germany) and placed in ice. An abdominal incision then was made and mice were perfused with 1% phosphate-buffered saline (PBS) to remove blood contaminants, and the liver, whole intestine, cecum, and visceral adipose were removed and immediately weighed. Intestines were divided into sections: small intestine, large intestine, and cecum. Each section was weighed whole and after removal of intestinal material. Cecal content was removed and weighed and samples were divided in half by weight. One half was dried overnight at 105°C and re-
weighed to determine dry matter content. The other half of the samples was processed immediately for short chain fatty acid (SCFA) analysis. Ileal sections were taken from small intestinal segments and sliced open length-wise. Ileal samples were fixed in 10% formalin and were processed by the Histology Lab at the College of Veterinary Medicine at the University of Illinois. Briefly, tissues were embedded in paraffin. One section was cut per paraffin block and sections were adhered to slides and stained using hematoxylin and eosin (H&E). Liver and ileum slides were photographed and images were captured using the Nanozoomer Digital Pathology System (Hamamatsu Photonics, Hamamatsu City, Japan).

Chemical analyses- Cecal contents were placed in micro-centrifuge tubes and acidified with 6.25% m-phosphoric acid and held at room temperature for 30 min after which they were frozen at -20°C overnight. Samples then were thawed and centrifuged at 13,000 x g for 10 min. Supernatant was transferred to gas chromatography vials and acetate, butyrate, propionate, valerate, isovalerate, and isobutyrate were measured by gas chromatography (28).

Diet and feces were analyzed for dry matter (DM), organic matter (OM), and ash (29). Total lipid content of feces was determined by acid hydrolysis followed by ether extraction according to the methods of the American Association of Cereal Chemists (30). Gross energy of fecal samples was determined by oxygen bomb calorimetry according to Parr Instrument Manuals (Parr Instrument Co., Moline, IL; No 203M, 205M, 207M, 246M).

Statistical analysis- Data analysis was conducted using Sigma Plot 11.2 (Systat Software, Chicago, IL). Food intake, energy intake, SCFA, and weight data were subjected to 1-way ANOVA to determine the main effect of diet. Post-hoc means separation using a Tukey adjustment was performed to determine differences among diet groups. When the test for normality failed (P<0.05), the Kruskal–Wallis one-way analysis of variance by ranks was performed followed by a Tukey adjustment in the case of equal groups and a Dunn’s adjustment when group size was not equal. Statistical significance was assumed at P<0.05. Data were presented as means ± SEM.
4.3 Results

Pectin delays weight gain when added to a high fat diet compared to cellulose at a 10% addition, but not less.

Figure 1 shows that pectin is protective against weight gain when added to a HFD at a level of 10%. Mice fed a HFD + 10% pectin (HFP) gained less weight than mice fed a HFD + 10% cellulose (HFC) after 12 wk of feeding (14.21g±1.44 vs. 24.81g±1.31, n=24-25, P<0.05).

Beginning at wk 1, mice fed HFC were heavier than mice fed LFC, LFP, and HFP (P<0.05). Beginning at wk 4, mice fed HFP weighed more than both LFD groups (P<0.05). HFP weighed less than HFC at all time points after baseline (P<0.05). Figure 2 shows that supplementation of 5% pectin to a HFD is not protective against weight gain. Mice were fed a HFD or LFD with the addition of 5% pectin (5P) or 5% cellulose (5C). By wk 3, both HFD groups (HF5C and HF5P) weighed more than LFD groups (LF5C and LF5P) (n=8, P<0.05). There was no significant difference in body weight between HF5C and HF5P or LF5C and LF5P at any time point. To determine if the total percent of fiber contributed to the prevention of weight gain and not the amount of pectin specifically, mice were fed a HFD or LFD with 5% pectin + 5% cellulose (PC) (Figure 3). HFPC slowed weight gain, but did not prevent weight gain to the same extent as HFP. HFC fed mice were significantly heavier than LFD fed mice after 3 wk of feeding, while HFPC mice were not significantly heavier than LFD groups until week 5 of feeding. After 12 wk of feeding, HFPC was not significantly different in weight than HFP or HFC (n=8).

Fructooligosaccharides and resistant starch do not protect against weight gain when added to a high fat diet.

To determine if weight gain was due to an intrinsic property of pectin or was due to the solubility or fermentation of the fiber, mice were placed on a HFD or LFD with 10% fructooligosaccharides (F) or 10% resistant starch (RS). Figure 2 shows mice fed HFF, HFRS, and HFC were s heavier than HFP beginning at wk 1 (P<0.05, n=8).

Pectin is not protective against weight gain in all knockout strains.

To explore the mechanism by which pectin affects weight gain, IL4-/-, IL1R1-/-, TLR4-/-, TLR2-/-, and MYD88-/- mice were fed a HFP, HFC, LFP, or a LFC diet for 12 wk (Table 4). In IL1R1-/- mice, there was no significant difference in weight among diet groups until wk 12 of feeding. At wk 12, HFC-fed mice were heavier than HFP and LFP groups (P<0.05). HFC-fed
IL4-/- mice were heavier than all other diet groups beginning at 3 wk of feeding (P<0.05). Pectin delayed weight gain in HFD-fed MYD88 -/- mice. After 6 wk of feeding, HFC-fed mice were heavier than LFP-fed mice (P<0.05). At 9 wk, HFC was heavier than both LFD groups, while there were no weight differences between HFP and any other group (P<0.05). At wk 12, HFP-fed mice were heavier than LFP-fed mice, while there was no difference in weight between HFP- and LFC- or HFC-fed mice (P<0.05). Pectin did not have an effect on weight in TLR4-/- or TLR2-/- mice. There were no significant differences in weight between TLR4-/- or TLR2-/- HFC- and HFP-fed mice at any time point. After 12 wk of feeding, HFD groups were heavier than LFD groups in both TLR4-/- and TLR2-/- mice (P<0.05).

**Pectin decreases hyperglycemia but not hyperinsulinemia in high fat diet-fed mice.**
Table 3 shows that fasting blood glucose concentrations were elevated in the HFC group compared to HFP, LFP, and LFC groups at 3 wk of feeding (n=8, P<0.05). There was no difference between HFD and LFD groups at 12 wk. The IPGTT data showed elevated glucose concentrations in the HFC group compared LFP and LFC (n=6, P<0.05). There was no difference in blood glucose concentrations between the HFP group and LFD groups, indicating a protective effect of pectin (Figure 5A). The IPITT data showed no difference in glucose concentration at any time point among diet groups (n=6) (Figure 5B).

**Pectin-fed mice consume more calories per day than cellulose-fed animals, while there was no difference in intake between resistant starch and fructooligosaccharide-fed animals.**
Figure 6 demonstrates food intake differences among diet groups. Average daily food intake was lower in HFC-fed mice compared to LFP-, LFC-, and HFP-fed mice (n=6, P≤0.02). Daily calorie intake of HFC-fed mice was less than LFC- and HFP-fed mice (P≤0.006). Average daily food intake was higher in LFRS- and LFF- fed mice than in HFRS- and HFF-fed mice (n=6, P≤0.03). There was no difference in calorie intake between RS- and F-fed mice regardless of fat content.

**Cecal SCFA and BCFA profiles differ among diets.**
Table 4 depicts the differences in cecal contents of SCFA and BCFA among diets. HFP-fed mice had 70% less cecal isobutyrate than LFC-fed mice (P≤0.05), 68% less cecal isovalerate than LFC-fed mice (P≤0.05), 56% less cecal isovalerate than LFP-fed mice (P≤0.05), 160% less
cecal valerate than LFC-fed mice (P≤0.05), and 134% greater cecal acetate than HFC-fed mice (P≤0.05). Cecal butyrate was 54% higher in LFP-fed mice compared to HFP-fed mice (P≤0.001), 73% higher than HFC-fed mice (P≤0.001), and 52% higher than LFC-fed mice (P≤0.001). There was 66% less cecal propionate in LFP-fed mice compared to HFC-fed mice (P≤0.05). HFF-fed mice had 68% less cecal isovalerate than LFF-fed mice (P≤0.05), 94% less than LFRS-fed mice (P≤0.05), and 70% less than HFRS-fed mice (P≤0.05). HFRS-fed mice had 101% less cecal propionate than LFRS-fed mice (P≤0.05), 134% less cecal propionate than LFF-fed mice, 338% less butyrate than LFF-fed mice (P≤0.05), and 245% less cecal butyrate than HFF-fed mice (P≤0.05). LFRS-fed mice had 47.2% less cecal valerate than HFRS-fed mice (P≤0.05).

Fecal lipid and energy content are greater in pectin-fed mice
Figure 7A shows that fecal lipid content was greater in pectin-fed mice compared to cellulose-fed mice (n=6, P≤0.001). Figure 7B shows fecal lipid content was greater in resistant starch-fed animals compared to fructooligosaccharide-fed animals (n=6, P≤0.001). Figure 7C shows fecal energy content was greater in HFP-fed mice compared to HFC-fed mice (n=6, P≤0.001).

Pectin reduces visceral fat and liver weight, and increases cecal weight and intestinal length
Table 5 shows pectin supplementation reduces accumulation of visceral fat. Weight of visceral fat from HFP-fed mice was not significantly different than HFC or LFD groups, while visceral fat in HFC-fed mice was greater than LFD-fed animals (n=7-8, P<0.05). HFP decreased liver weight compared to HFC (n=7-8, P=0.04). Pectin also increased the weight of the cecum (empty) compared to cellulose (n=7-8, P<0.05). Pectin increased cecal content weight in both HFP and LFP, but the difference was only significant between LFP vs. HFC (n=7-8, P<0.05). LFP increased total intestinal length compared to other diets (n=7-8, P≤0.04).

4.4 Discussion
Several rodent studies have demonstrated a protective effect of soluble fiber against weight gain (2, 3, 13, 15, 16, 31). In support of these findings, we found that animals fed a HFD supplemented with pectin gained less weight than mice fed a cellulose-supplemented diet. Pectin supplementation also resulted in decreased accumulation of visceral fat compared to cellulose.
Hove *et al.* found pectin to decrease both weight and food intake in rats when added to both a low protein and high protein diet, while cellulose supplementation increased weight and food intake only when added to a low protein diet (31). Surprisingly, in this study, mice fed a HFD + cellulose (HFC) had decreased energy intake compared to LFD + cellulose (LFC)- and HFD + pectin (HFP)-fed mice. This could be an artifact of study design, as HFC-fed mice were significantly heavier than other diet groups and may have been more uncomfortable on wire bottom cages resulting in anxiety and decreased food intake. This is not likely the case as weight gain on resistant starch and fructooligosaccharides treatments did not alter calorie intake. Interestingly, pectin was only protective at the level of 10%. Supplementation of 5% pectin with or without the addition of 5% cellulose was not effective in prevention of weight gain. This may indicate a dose-response effect, as pectin is only effective in weight prevention at higher doses. Unfortunately, the weight benefit from pectin noted in rodents may not carry over to humans. In a clinical trial, 4 wk supplementation of 15 g/d of pectin did not decrease weight compared to 12 g/d of cellulose (32). This may be due to the dose of pectin administered in this study. Supplementation of some DFs may not be able to be delivered at doses high enough to be effective in humans. Intake of large amounts of soluble DF may result in rapid fermentation and excessive production of gases leading to discomfort and gastrointestinal distress (33).

To determine if pectin’s delay of weight gain in HFD-fed mice was due solely to the solubility of pectin, we explored the effect two additional soluble fibers, resistant starch and fructooligosaccharides, on the prevention of weight gain. Both are fermentable fibers, but fructooligosaccharides are quickly fermented, while resistant starch is slow to degrade. Mice fed a HFD supplemented with either 10% fructooligosaccharides or 10% resistant starch exhibited a similar pattern of weight gain to mice supplemented with 10% cellulose. There was no difference in energy intake between fructooligosaccharide and resistant starch diet groups. This suggests that the ability of pectin to delay weight gain in combination with a HFD may extend beyond solubility and ability to delay gastric emptying.

Reduced weight gain in HFP-fed mice compared to HFC-fed mice may be due, in part, to increased fecal lipid excretion. This study supports previous findings of increased fecal lipid excretion after pectin supplementation in both animals and humans (34-36). In a human study,
15 g of citrus pectin for 3 wk increased fecal lipid excretion by 44% (35). In fact, pectin is capable of binding 4 times its weight in lipid through hydrogen bonding of methoxy-carbonyl groups (37). Extent of lipid binding is dependent on the degree of methylation of pectin. The dose-response effect of pectin on weight reduction may be related to lipid-binding capacity. The larger percentage of pectin in the diet, the larger capacity of pectin to bind lipids.

The prevention of weight gain by pectin may not be solely due to increased lipid binding capacity. In ILIR1, IL-4, TLR2, TLR4, and MYD88 knockout mice fed a HFD supplemented with pectin and cellulose, pectin was not equally protective in the prevention of weight gain. IL-1, IL-4, and members of the TLR/MyD88 signaling pathway may all play a role in weight regulation. Dis-regulation of the pro-inflammatory cytokine, IL-1, has been shown to result in weight modulation (38, 39). IL1RA (IL-1 receptor antagonist)-deficient mice have decreased fat mass compared to wild type control mice (38) while IL1R1 (IL-1 receptor)-deficient mice (IL1R1/-) are prone to late onset obesity compared to controls (39). Here, IL1R1-deficient mice fed a HFD or LFD supplemented with pectin or cellulose did not differ in weight until wk 12. At wk 12, HFC-fed mice were significantly heavier than HFP- and LFD-fed groups. Weight differences between HFC and LFD groups occur by 3 wk in wild type mice. Decreased functional IL-1 may initially be protective against diet-induced obesity. Although Garcia et al. found IL1R1/- mice to be at risk for the development of obesity, weight differences between IL1R1/- mice and controls were not seen until after 4 mo of age (39). A second pro-inflammatory cytokine, IL-4, also has been implicated in the pathogenesis of obesity. Administration of IL-4 in combination with a HFD feeding exacerbates weight gain in rats (40). In our study, IL-4-deficient mice fed a HFC diet became significantly heavier than HFP- and LFD-fed mice at 3 wk of feeding and followed a similar weight gain pattern to wild type mice. Therefore, the protective effect of pectin does not appear to involve a IL-1 or IL-4 mechanism.

Members of the toll-like receptor (TLR) signaling pathway also have been linked to obesity. There are 10 members of the TLR family in humans (41). TLR4 is a receptor located on monocytes that detects lipopolysaccharide (LPS). In wild type mice, HFD-feeding increases blood monocytes, but decreases surface TLR4 expression (42). TLR4-deficient (TLR4/-) mice have been found to maintain low body fat regardless of diet (43). In contrast, this study found
HFD-fed mice to be significantly heavier than LFD-fed mice, regardless of fiber content beginning after 3 wk of feeding. Interestingly, pectin was not protective against weight gain in the TLR4-/- mice, suggesting the TLR4 receptor may be necessary for pectin to reduce weight gain. TLR2 deficient (TLR2-/-) mice also have been shown to be protected from HFD-induced weight gain (44). In this study, although differences in weight among diet groups did not occur until 6 wk of feeding, by wk 12, HFD groups were significantly heavier than LFD groups, suggesting the TLR2 receptor also may play a role in reduction of weight gain by pectin.

TLR4 and TLR2 are components of the MyD88-dependent signaling pathway (41). MyD88 also has been reported to be essential in the progression of diet-induced obesity. CNS-specific MyD88 knockout mice (MyD88-/-) are protected from HFD-induced weight gain (45). Here, MyD88 deficiency was found to delay weight gain in HFD-fed mice. HFC-fed MyD88-/- mice were not significantly heavier than LFD-fed MyD88-/- mice until wk 9. At wk 9, HFP-fed mice were not significantly different from either HFC- or LFD-fed mice, indicating that although pectin may offer some protection, MyD88-/- also may be a part of the mechanism of inhibition of weight gain by pectin.

In addition to conferring a weight benefit, DF improves glycemic control, fasting blood glucose, and insulin sensitivity by altering carbohydrate absorption through the formation of viscous gels and inhibition of glucose transport across the intestinal membrane (1, 46, 47). Here, 10% pectin supplementation slowed HFD-induced elevation in fasting blood glucose concentrations after an 8 h fast. Pectin supplementation also improved glucose tolerance compared to cellulose, but did not affect insulin tolerance. This may indicate pectin supplementation prevents or slows the pre-diabetic state noted in cellulose-supplemented mice. The ability of soluble fiber to lower blood glucose is inconsistent in animal studies. Psyllium and sugar cane fiber decreased fasting blood glucose concentrations in rodents, while apricot fiber and okara did not (21, 48, 49). In human studies, several soluble fibers including pectin and guar gum improved oral glucose tolerance (50). Soluble β-glucan improved glucose tolerance and in contrast to our findings, decreased serum insulin concentrations after supplementation at a 2 and 4% level (2). Inconsistencies in results may be due to type of fiber administered and dosage of DF. DF varies in intrinsic characteristics that may result in differences in physiologic effect.
Prevention of diabesity by DF may be due, in part, to production of SCFAs. Fermentable DFs are partially or completely fermented in the intestine to SCFAs: butyrate, propionate, and acetate (51). In humans, pectin supplementation increased total SCFAs compared to cellulose (52). Here, pectin increased the cecal concentration of acetate, propionate, and butyrate compared to cellulose. In contrast, in a study in rats, pectin increased acetate, but not butyrate or propionate (53). This discrepancy may be due to study design Fukunaga et al. administered pectin in an enteral formula as opposed to a component in a pellet-based diet. Resistant starch and fructooligosaccharide groups did not differ in cecal acetate concentration, but had significant differences in concentrations of cecal propionate and butyrate. This is consistent with other studies which found that fructooligosaccharides and resistant starch increased butyrate and decreased acetate (33). SCFAs may affect weight and diabetes-related outcomes by modifying the expression of key genes involved in lipid and carbohydrate metabolism (54-57). As an example, propionate decreased fatty acid and cholesterol synthesis by blocking the incorporation of acetic acid into fats and sterols (62). Furthermore, DF intake alters microbiota populations that can directly impact lipid and cholesterol metabolism by enhancing bile acid deconjugation (58, 59). Fermentable DF intake also has been shown to alter genes and hormones related to carbohydrate metabolism such as pro-glucagon and glucagon-like peptide 1 (13, 23, 60). This may occur as a result of the influence on DF on intestinal morphology, specifically augmentation of L-cells (13).

Intake of DF also has trophic effects on the intestine, altering ileum length and weight and changing the morphology of the small intestine (53, 61, 62). Both pectin and cellulose have been shown to increase villus height and width compared to animals fed a control diet (62, 63). Pectin, in particular, has been shown to increase villus length compared to cellulose (61). In chickens, diets supplemented with pectin have been shown to increase the percentage of tongue-shaped villi and decrease the shape of ridge-shaped villi in the ileum compared to non-pectin supplemented diets (64). Compared to a fiber-free diet, pectin significantly increased length and weight of the small intestine, cecum, and colon in rats fed an enteral diet for 4 wk (53). The extent of these trophic effects also may depend on degree of methylation. Here, pectin significantly increased total intestinal length. Trophic effects of pectin could be the result of increased brush border enzymes or due to changes in SCFA production. Pectin has been shown
to increase enteroglucagon and glucagon-like peptide-2, both of which have been shown to have trophic effects on the intestine (53).

In addition to changes in the morphology of the intestine, DF supplementation of rodents decreased adiposity and liver weight (2, 49, 65). β-glucan has been shown to decrease liver and adipose tissue weight compared to control-fed animals (2). Here, pectin reduced fat accumulation compared to cellulose. HFC-fed mice had greater visceral fat compared to LFD-fed groups, while there was not a significant difference in amount of visceral fat between HFP-fed mice and LFD-fed groups.

Not all soluble DFs are equal. This study supports current research that shows contradictions in the beneficial effects of fiber, particularly as regards weight reduction. Pectin prevented weight gain in HFD-fed mice, while resistant starch and fructooligosaccarides, both soluble and fermentable fibers, did not. Differences among DF could be due to fermentation patterns or lipid binding capacity. Perhaps more interestingly, this study suggests that toll-like receptor signaling may be involved in both delaying diet induced obesity and the mechanism by which pectin protects against weight gain.
### 4.5 Tables and Figures

<table>
<thead>
<tr>
<th>Diet</th>
<th>Kcal/g *</th>
<th>Fat</th>
<th>Protein</th>
<th>CHO</th>
<th>Cellulose</th>
<th>Pectin</th>
<th>FOS</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat 5% cellulose (HF5C)</td>
<td>5.24</td>
<td>34.9(60)</td>
<td>26.2(20)</td>
<td>26.3 (20)</td>
<td>50(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat 5% pectin (HF5P)</td>
<td>5.23</td>
<td>34.8(60)</td>
<td>26.1 (20)</td>
<td>26.3 (20)</td>
<td></td>
<td>52.5(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat pectin/cellulose (HFPC)</td>
<td>4.91</td>
<td>32.7(60)</td>
<td>24.5 (20)</td>
<td>24.7 (20)</td>
<td>50(0)</td>
<td>52.5(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat pectin (HFP)</td>
<td>4.89</td>
<td>32.6(60)</td>
<td>24.5(20)</td>
<td>24.6(20)</td>
<td></td>
<td></td>
<td>105(0)</td>
<td></td>
</tr>
<tr>
<td>High fat cellulose (HFC)</td>
<td>4.89</td>
<td>32.6(60)</td>
<td>24.5(20)</td>
<td>24.6(20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat FOS (HFF)</td>
<td>4.89</td>
<td>32.6(60)</td>
<td>24.5(20)</td>
<td>24.6(20)</td>
<td></td>
<td></td>
<td></td>
<td>105(0)</td>
</tr>
<tr>
<td>High fat RS (HFRS)</td>
<td>4.89</td>
<td>32.6(60)</td>
<td>24.5(20)</td>
<td>24.6(20)</td>
<td></td>
<td></td>
<td></td>
<td>105(0)</td>
</tr>
<tr>
<td>Low fat 5% cellulose (LF5C)</td>
<td>3.85</td>
<td>4.3 (10)</td>
<td>19.2(20)</td>
<td>67.3(70)</td>
<td>50(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat 5% pectin (LF5P)</td>
<td>3.84</td>
<td>4.3 (10)</td>
<td>19.2(20)</td>
<td>67.1(70)</td>
<td></td>
<td></td>
<td>52.5(0)</td>
<td></td>
</tr>
<tr>
<td>Low fat pectin/cellulose (LFPC)</td>
<td>3.66</td>
<td>4.1 (10)</td>
<td>18.3(20)</td>
<td>64.1(70)</td>
<td>50(0)</td>
<td></td>
<td>52.5(0)</td>
<td></td>
</tr>
<tr>
<td>Low fat pectin (LFP)</td>
<td>3.65</td>
<td>4.1 (10)</td>
<td>18.3(20)</td>
<td>64.0(70)</td>
<td></td>
<td></td>
<td>105(0)</td>
<td></td>
</tr>
<tr>
<td>Low fat cellulose (LFC)</td>
<td>3.67</td>
<td>4.1 (10)</td>
<td>19.2(20)</td>
<td>67.3(70)</td>
<td></td>
<td></td>
<td>100(0)</td>
<td></td>
</tr>
<tr>
<td>Low fat FOS (LFF)</td>
<td>3.85</td>
<td>4.3 (10)</td>
<td>18.3(20)</td>
<td>64.0(70)</td>
<td>50(0)</td>
<td></td>
<td>105(0)</td>
<td></td>
</tr>
<tr>
<td>Low fat RS (LFRS)</td>
<td>3.65</td>
<td>4.1 (10)</td>
<td>18.3(20)</td>
<td>64.0(70)</td>
<td></td>
<td></td>
<td>105(0)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4:** Macronutrient composition of research diets. Values for fat, protein, carbohydrate, and dietary fiber are presented as g/4057 kcal (% kcal). CHO= carbohydrate, FOS= fructooligosaccharides, RS= resistant starch. *conversion factors used: 4 for protein, 4 for carbohydrates, and 9 for fat.
**Figure 9:** 10% pectin slows weight gain.

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P) or 10% cellulose (C). * denotes significant differences in weight between HFC- and HFP-fed mice (P<0.05, n=24-25).
Figure 10: 5% pectin was not protective against weight gain

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 5% pectin (5P) or 5% cellulose (5C). Mice were weighed weekly. *indicates a significant difference between HF and LF diets (n=8, P<0.05).
Figure 11: A combination of pectin and cellulose did not protect against weight gain to the same extent as 10% pectin.

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P), 10% cellulose (C), or 5% pectin + 5% cellulose (PC). * indicates point at which HFC fed mice became heavier than all 3 LFD groups. # indicates the point at which HFCP became heavier than all 3 LFD groups. Mice were weighed weekly (n=8, P<0.001).
Figure 12: Resistant starch and fructooligosaccharides are not protective against weight gain.

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P), 10% cellulose (C), 10% resistant starch (RS), or 10% fructooligosaccharides (F). Mice were weighed weekly. *denotes the time point where HFRS-, HFF-, and HFC-fed mice became significantly heavier than HFP fed mice (n=8, \(P<0.05\)).
Knockout    Diet  Baseline  3 weeks  6 weeks  9 weeks  12 weeks
IL-4/-

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFP</td>
<td>11.6±0.9</td>
<td>22.5±0.3a</td>
<td>26.1±0.6a</td>
<td>27.3±0.8a</td>
<td>29.0±0.8ab</td>
</tr>
<tr>
<td></td>
<td>LFC</td>
<td>14.3±1.4</td>
<td>22.4±0.7a</td>
<td>25.1±0.6a</td>
<td>26.8±1.2a</td>
<td>26.8±0.6a</td>
</tr>
<tr>
<td></td>
<td>HFP</td>
<td>11.4±0.6</td>
<td>22.1±0.7a</td>
<td>26.1±1.0a</td>
<td>30.2±1.0a</td>
<td>31.7±1.3b</td>
</tr>
<tr>
<td></td>
<td>HFC</td>
<td>14.7±1.2</td>
<td>25.2±0.6b</td>
<td>30.4±1.0b</td>
<td>35.0±1.4b</td>
<td>39.3±2.0c</td>
</tr>
</tbody>
</table>

IL1R1/-

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFP</td>
<td>14.8±1.4</td>
<td>22.5±0.5</td>
<td>25.5±0.4</td>
<td>27.4±0.4</td>
<td>28.8±0.4a</td>
</tr>
<tr>
<td></td>
<td>LFC</td>
<td>15.0±0.7</td>
<td>22.8±1.2</td>
<td>25.3±1.3</td>
<td>28.8±1.3</td>
<td>29.9±1.7ab</td>
</tr>
<tr>
<td></td>
<td>HFP</td>
<td>14.2±2.3</td>
<td>21.9±1.1</td>
<td>24.7±0.7</td>
<td>26.9±0.5</td>
<td>28.0±0.8a</td>
</tr>
<tr>
<td></td>
<td>HFC</td>
<td>14.3±1.5</td>
<td>24.6±1.0</td>
<td>30.2±1.3</td>
<td>32.6±1.7</td>
<td>37.3±2.2b</td>
</tr>
</tbody>
</table>

TLR4/-

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFP</td>
<td>7.3±0.6</td>
<td>15.9±1.2a</td>
<td>18.9±1.3a</td>
<td>21.2±0.9a</td>
<td>22.0±0.9a</td>
</tr>
<tr>
<td></td>
<td>LFC</td>
<td>9.5±0.8</td>
<td>18.8±1.1ab</td>
<td>21.2±1.1ac</td>
<td>23.8±0.8ab</td>
<td>24.0±1.3a</td>
</tr>
<tr>
<td></td>
<td>HFP</td>
<td>8.4±0.9</td>
<td>19.7±0.7b</td>
<td>23.0±0.9b</td>
<td>25.1±1.0b</td>
<td>28.0±0.8b</td>
</tr>
<tr>
<td></td>
<td>HFC</td>
<td>7.9±1.1</td>
<td>18.9±0.7ab</td>
<td>22.9±0.7bc</td>
<td>25.8±0.5b</td>
<td>28.7±0.6b</td>
</tr>
</tbody>
</table>

TLR2/-

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFP</td>
<td>9.6±0.4</td>
<td>17.7±0.6</td>
<td>20.4±0.5a</td>
<td>21.8±0.6a</td>
<td>22.4±0.5a</td>
</tr>
<tr>
<td></td>
<td>LFC</td>
<td>9.7±0.9</td>
<td>18.3±1.1</td>
<td>21.7±0.6ab</td>
<td>23.4±0.7ab</td>
<td>24.4±0.6a</td>
</tr>
<tr>
<td></td>
<td>HFP</td>
<td>10.5±1.2</td>
<td>18.9±0.6</td>
<td>22.6±0.9ab</td>
<td>25.8±1.1bc</td>
<td>27.6±1.1b</td>
</tr>
<tr>
<td></td>
<td>HFC</td>
<td>9.9±1.9</td>
<td>20.4±0.7</td>
<td>24.2±0.8b</td>
<td>27.5±1.1c</td>
<td>29.8±1.1b</td>
</tr>
</tbody>
</table>

MyD88/-

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFP</td>
<td>14.4±1.5ab</td>
<td>20.2±0.9</td>
<td>22.2±0.8a</td>
<td>24.5±0.9a</td>
<td>24.8±0.9a</td>
</tr>
<tr>
<td></td>
<td>LFC</td>
<td>16.1±1.8a</td>
<td>21.9±1.0</td>
<td>24.2±0.9ab</td>
<td>26.1±0.8a</td>
<td>27.4±0.8ab</td>
</tr>
<tr>
<td></td>
<td>HFP</td>
<td>10.6±1.3b</td>
<td>19.7±1.3</td>
<td>24.1±1.0ab</td>
<td>27.9±1.0ab</td>
<td>30.7±1.1bc</td>
</tr>
<tr>
<td></td>
<td>HFC</td>
<td>11.5±0.4b</td>
<td>21.2±0.8</td>
<td>27.0±1.6b</td>
<td>31.2±2.7b</td>
<td>34.2±1.9c</td>
</tr>
</tbody>
</table>

**Table 5:** Pectin is not protective against weight gain in all knockout strains.

IL1R1/-, IL4/-, TLR4/-, TLR2/-, and MYD88/- mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P) or 10% cellulose (C). Mice were weighed weekly. Different letters represent significant differences among diet groups. Values are presented as means ± SEM. P<0.05. IL1R1/-: n=8-12; IL4/- n=8-10; MYD88/- n=8-12; TLR4/- n=7-12; TLR2/- n=8-11.
Table 6: Pectin blunts high fat diet-induced hyperglycemia

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P) or 10% cellulose (C). Blood samples were collected from the tail after 8 h of fasting. Fasting blood glucose was measured weekly. Values presented as means ± SEM. Letters indicate significant differences (n=8, P≤0.05). LFP= 10% fat + 10% pectin, LFC= 10% fat + 10% cellulose, HFP= 60% fat + 10% pectin, HFC= 60% fat + 10% cellulose.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dL</td>
<td>92.8±14.2a</td>
<td>109.9±18.9</td>
<td>103.6±14.8a</td>
<td>94.4±9.5a</td>
<td>106.7±7.1a</td>
<td>113.1±5.1a</td>
</tr>
<tr>
<td></td>
<td>126.5±12.6ab</td>
<td>145.8±16.5</td>
<td>117.3±18.7a</td>
<td>110.5±3.1a</td>
<td>158.8±14.4ab</td>
<td>114.6±5.1a</td>
</tr>
<tr>
<td></td>
<td>141.4±7.4b</td>
<td>111.1±25.5</td>
<td>124.0±19.7a</td>
<td>160.0±7.3b</td>
<td>190.6±8.4bc</td>
<td>142.8±7.1b</td>
</tr>
<tr>
<td></td>
<td>129.9±5.8ab</td>
<td>112.9±21.5</td>
<td>208.6±19.2b</td>
<td>153.3±4.3b</td>
<td>224.6±26.6c</td>
<td>139.5±5.7b</td>
</tr>
</tbody>
</table>
Figure 13: *Pectin prevents hyperglycemia but not insulinemia*

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P) or 10% cellulose (C). Both the IPGTT and IPITT were performed after 12 wk of diet feeding. (A) IPGTT was performed after a 4 h fast. (B) IPITT was performed on fed mice. Values presented as means ± SEM. LFP= 10% fat + 10% pectin, LFC= 10% fat + 10% cellulose, HFP= 60% fat + 10% pectin, HFC= 60% fat + 10% cellulose. Different letters indicate significant differences among treatments (n=6, P<0.05).
Figure 14 (A-D): *High fat pectin-fed animals have a greater energy intake than cellulose-fed animals*

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P), 10% cellulose (C), 10% resistant starch (RS), or 10% fructooligosaccharides (F). After 10 wk of feeding, mice were placed in metabolic cages and food intake was recorded daily for the first 6 d (A, B). Energy intake was calculated from daily food intake (C, D). Different letters indicate significant differences (n=6, P≤0.04).
<table>
<thead>
<tr>
<th>Diet</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Total SCFA</th>
<th>Isobutyrate</th>
<th>Isovalerate</th>
<th>Valerate</th>
<th>Total BCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LFP</td>
<td>241.3±27.5a</td>
<td>28.9±2.6a</td>
<td>52.2±6.8a</td>
<td>171.9±66.2a</td>
<td>2.9±0.4ab</td>
<td>2.1±0.2a</td>
<td>1.7±0.2a</td>
<td>31.4±12.3a</td>
</tr>
<tr>
<td>LPC</td>
<td>147.6±16.1ab</td>
<td>18.1±1.8ab</td>
<td>25.1±2.3b</td>
<td>101.8±39.7ab</td>
<td>3.4±0.5a</td>
<td>2.9±0.5a</td>
<td>3.1±0.5a</td>
<td>18.4±7.1ab</td>
</tr>
<tr>
<td>HFP</td>
<td>239.6±66.2a</td>
<td>16.7±2.2ab</td>
<td>24.1±2.6b</td>
<td>149.5±57.9b</td>
<td>1.7±0.3b</td>
<td>0.9±0.06b</td>
<td>1.2±0.2b</td>
<td>14.8±5.7b</td>
</tr>
<tr>
<td>HFC</td>
<td>105.3±39.5b</td>
<td>9.9±3.4b</td>
<td>13.8±3.1b</td>
<td>68.8±41.0ab</td>
<td>2.5±0.8ab</td>
<td>2.0±0.7ab</td>
<td>1.9±0.6ab</td>
<td>10.8±5.4b</td>
</tr>
<tr>
<td>LFRS</td>
<td>210.9±19.5</td>
<td>41.6±4.9a</td>
<td>42.2±11.3ab</td>
<td>294.8±26.4a</td>
<td>2.5±0.4</td>
<td>3.8±0.5</td>
<td>4.9±6.6</td>
<td>11.2±1.4</td>
</tr>
<tr>
<td>LFF</td>
<td>194.3±30.2</td>
<td>48.3±10.4a</td>
<td>88.3±20.4a</td>
<td>330.9±57.5a</td>
<td>1.9±0.5</td>
<td>3.3±0.7</td>
<td>5.6±1.2a</td>
<td>10.9±2.3</td>
</tr>
<tr>
<td>HFRS</td>
<td>129.4±12.9</td>
<td>20.7±2.9b</td>
<td>20.2±1.8b</td>
<td>170.2±15.8b</td>
<td>2.7±0.4</td>
<td>3.3±0.5</td>
<td>2.6±0.2b</td>
<td>8.6±1.0</td>
</tr>
<tr>
<td>HFF</td>
<td>185.4±32.0</td>
<td>27.6±5.8ab</td>
<td>69.6±18.8a</td>
<td>282.6±57.1a</td>
<td>1.3±0.3</td>
<td>2.0±0.4</td>
<td>2.5±0.6ab</td>
<td>5.7±1.1</td>
</tr>
</tbody>
</table>

**Table 7:** Cecal short-chain fatty acid profiles after 12 weeks of fiber supplementation.

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P), 10% cellulose, 10% resistant starch (RS), or 10% fructooligosaccharides (F) for 12 wk. Different letters indicate significant differences among treatments. (n=8, P≤0.05) Values are presented as mean ± SEM.
Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P), 10% cellulose (C), 10% fructooligosaccharides (F), or 10% resistant starch (RS). After 10 wk of feeding, mice were placed in metabolic cages and feces were collected for 7 d. % acid hydrolyzed fat (AH) and total energy was measured in fecal samples (n=6, P≤0.001). A) % AH fat in fecal samples from P- and C-fed mice. B) % AH fat in fecal samples from F- and RS-fed mice. C) Fecal energy content in P- and C-fed mice. Different letters indicate significant differences.

**Figure 15:** High fat pectin-fed animals have a greater fecal lipid and fecal energy content then cellulose-fed animals
Table 8: *Pectin reduces adiposity, reduces liver weight, and has a trophic effect on the intestine*

Mice were placed on a high fat (HF) or a low fat (LF) diet containing either 10% pectin (P) or 10% cellulose. After 12 wk of feeding, mice were sacrificed and organs were harvested.

Different letters indicate significant differences among treatments (n=8, P≤0.05). Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Visceral fat (g)</th>
<th>Liver (g)</th>
<th>Cecum (g)</th>
<th>Cecal contents (g)</th>
<th>Int. length (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFP</td>
<td>25.3±0.8a</td>
<td>0.1±0.0a</td>
<td>1.2±0.1a</td>
<td>0.2±0.0a</td>
<td>0.6±0.0a</td>
<td>51.3±1.8a</td>
</tr>
<tr>
<td>LFC</td>
<td>27.0±0.8ab</td>
<td>1.2±0.1a</td>
<td>1.1±0.1a</td>
<td>0.1±0.0b</td>
<td>0.3±0.1ab</td>
<td>38.9±0.8b</td>
</tr>
<tr>
<td>HFP</td>
<td>31.4±1.3b</td>
<td>2.0±0.4ab</td>
<td>1.1±0.0a</td>
<td>0.2±0.0a</td>
<td>0.5±0.0ab</td>
<td>44.0±1.1b</td>
</tr>
<tr>
<td>HFC</td>
<td>43.9±1.6c</td>
<td>5.4±0.3b</td>
<td>1.6±0.1b</td>
<td>0.1±0.0b</td>
<td>0.2±0.1b</td>
<td>39.2±2.7b</td>
</tr>
</tbody>
</table>
4.6 References


CHAPTER 5
Summary and Discussion

The main findings from this study support increasing epidemiologic reports of negative associations between obesity and cognition. A high fat diet (HFD) induced anxiety-like behavior, increased reward-seeking, and decreased both spatial and non-spatial memory after only 1 week of feeding. HFD-induced memory deficit was reversed by ceasing HFD feeding, indicating a transient effect of the diet. Additionally, short-term HFD feeding disrupted brain-derived neurotrophic growth factor (BDNF) and nerve growth factor (NGF), growth factors involved in neuronal plasticity and the dopaminergic pathway in the cortex. Administration of a dopamine transport inhibitor, methylphendidate, restored non-spatial memory, while administration of anti-depressants did not. More studies are being published suggesting a common mechanism between obesity and attention deficit disorder. Both obesity and attention deficit disorder may involve alterations in the dopamine reward system. Further research is needed to explore the role of the HFD in these conditions. BDNF is closely related to the dopaminergic pathway and dopaminergic functions. Dopamine agonists have been shown to regulate expression of BDNF, indicating dopamine is a regulator of BDNF in the prefrontal cortex. In this study, we have demonstrated a decrease in BDNF expression in the cortex of HFD fed mice after 1 week of feeding suggesting dopamine levels may also be altered, as a decreased in dopamine would lead to a decrease in BDNF expression. Dopamine levels in the cortex are thought to be related to cognition, while dopamine levels in the striatum are thought to be related to motivation. To confirm the role of dopamine in memory deficit and reward-seeking behavior noted in this study, the amount of dopamine in the pre-frontal cortex and striatum in HFD- and LFD-fed mice could be measured using HPLC methods. Decreases in dopamine in the cortex of HFD-fed mice compared to LFD-fed mice would lend evidence to the hypothesis that decreased dopamine is a contributing factor to the non-spatial and spatial memory deficit seen in HFD-fed mice. Altered expression of dopamine receptor 2 (DRD2) and dopamine receptor 4 (DRD4) in striatum and prefrontal cortex, respectively, have been associated with altered dopamine transport activity as well as psychiatric disorders such as ADHD. DRD2 has been associated with motivational activity, while deficiency of DRD4 had been linked to impaired memory. Expression of dopamine receptor 1 (DRD1) is especially high
in the prefrontal cortex and is the most abundant dopamine receptor found in this region. Stimulation of DRD1 and DRD2 receptors with a dopamine agonist in vitro increased NGF. In addition, DRD1 receptors have been found to be involved in object recognition. To determine whether a HFD alters the expression of dopamine receptors in the cortex and striatum, DRD1, DRD2, and DRD4 mRNA expression could be measured in the cortex and striatum of HFD- and LFD-fed mice after 1 and 3 weeks of feeding. Alternately, dopamine receptor knock-out mice could be used.

Additionally, this study found that pectin is protective against diet-induced obesity in a dose-dependent matter. Pectin also was found to be protective against hyperglycemia. Resistant starch and fructooligosaccharides, soluble, fermentable fibers, were not protective against weight gain. In fact, they shared a similar weight gain pattern to cellulose, a non-digestible, non-fermentable fiber. Interestingly, pectin was not protective against weight gain in TLR4-/- and TLR2-/- mice, suggesting the protective effect of pectin, may involve the TLR signaling pathway. In wild-type mice, pectin appeared to protect against weight gain my increasing fecal lipid excretion. To further explore the mechanism by which pectin protects against weight gain, fecal lipid excretion could be examined in TLR4-/- mice. TLR4-/- mice are to some extent, immune to weight gain. It is possible that TLR4-/- mice have increased fecal lipid excretion similar to pectin-fed HFD mice. The effect of pectin on weight reduction could also be the result of communication through fatty acid receptors. To test this hypothesis, fatty acid receptor knockout mice could be fed the DF high fat diets to determine if pectin would still be protective if the fatty acid receptors were not functional.

These studies demonstrated the detrimental effect of a HFD on memory and cognition and the protective effect of pectin on diabesity. An interesting study would be to test memory and cognition in juvenile mice receiving a HFD supplemented with pectin to determine if pectin is also protective against HFD-induced memory deficit.