Effects of the Antihistamine Fexofenadine on Body Fat, Glucose and Body Weight: Impact of Dietary Fat Type and Pre-Drug Run-in Period

Elisabeth C. Smith

Follow this and additional works at: https://knowledge.library.iup.edu/etd

Part of the Biology Commons, Nutrition Commons, and the Public Health Commons

Recommended Citation
https://knowledge.library.iup.edu/etd/1470

This Thesis is brought to you for free and open access by Knowledge Repository @ IUP. It has been accepted for inclusion in Theses and Dissertations (All) by an authorized administrator of Knowledge Repository @ IUP. For more information, please contact cclouser@iup.edu, sara.parme@iup.edu.
EFFECTS OF THE ANTIHISTAMINE FEXOFENADINE ON
BODY FAT, GLUCOSE AND BODY WEIGHT: IMPACT OF
DIETARY FAT TYPE AND PRE-DRUG RUN-IN PERIOD

A Thesis
Submitted to the School of Graduate Studies and Research
in Partial Fulfillment of the
Requirements for the Degree
Master of Science

Elisabeth C. Smith
Indiana University of Pennsylvania
May 2017
Indiana University of Pennsylvania
School of Graduate Studies and Research
Department of Biology

We hereby approve the thesis of

Elisabeth C. Smith

Candidate for the degree of Master of Science

________________________________________
Daniel Widzowski, Ph.D.
Assistant Professor of Biology, Advisor

________________________________________
Robert Hinrichsen, Ph.D.
Professor of Biology

________________________________________
Thomas Simmons, Ph.D.
Professor of Biology

________________________________________
Idamarie Laquatra, Ph.D.
Assistant Professor of Food and Nutrition

ACCEPTED

________________________________________
Randy L. Martin, Ph.D.
Dean
School of Graduate Studies and Research
Both high fat diets and antihistamines have been implicated in human and animal metabolic disorders. Interaction of these factors is poorly understood. Omega-3 hepatoprotection is under-studied regarding drug-diet interactions. Two murine studies examined high fat versus omega-3 fatty acid diets and fexofenadine administration on metabolic disturbances. Based on run-in period, fexofenadine altered body weights specific to dietary grouping. With longer run-in times, fexofenadine and high-fat diet increased fat pad, liver, and body weights, while omega-3 body weights matched control. In contrast, shorter run-in times resulted in body weight decreases in high fat and omega-3-fed fexofenadine-treated mice, as well as increased fat pad masses and reduced serum glucose concentrations. Fexofenadine administration appears to affect body weight dependent on dietary fat and pre-drug run-in periods. Stress-induced biological factors may play a role in acclimation of mice to study conditions.
ACKNOWLEDGMENTS

I would like to express my utmost appreciation for the support and guidance given me by my advisor and thesis committee chair, Dr. Daniel Widzowski. Having had the opportunity to work under his supervision has provided me insight into his degree of expertise and level of enthusiasm that renders his leadership invaluable me as well as to many others. His tireless commitment to the betterment of others has been and continues to be an inspiration.

I would also like to thank my thesis committee members -- Dr. Robert Hinrichsen, Dr. Thomas Simmons, and Dr. Idamarie Laquatra for their guidance, eagerness to provide assistance, and intentionality in offering feedback for the furtherance of my academic aptitude. They have proven to be most stellar in delivering direction, advice, and leadership. I am grateful for them.

The selfless giving of time from fellow lab members, especially during data collection, was invaluable to me. These undergraduate colleagues clearly have promising prospects based both on their dedication to and enthusiasm for interpersonal collaboration as well as the advancement of scientific methodology -- all dosed with a good bit of humor.

Last, but not least, I would like to thank my husband and children for their unfailing support and encouragement throughout the process of two part-time graduate degrees. Without their help, well... let’s just say it could have gotten ugly.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>INTRODUCTION ................................................................. 1</td>
</tr>
<tr>
<td></td>
<td>Objectives of Study ................................................................. 4</td>
</tr>
<tr>
<td></td>
<td>Hypothesis ................................................................. 4</td>
</tr>
<tr>
<td>II</td>
<td>LITERATURE REVIEW ................................................................. 5</td>
</tr>
<tr>
<td></td>
<td>The Role of Diet in NAFLD ................................................................. 8</td>
</tr>
<tr>
<td></td>
<td>H1 Antihistamine Use and Metabolic Disturbances ................................................................. 13</td>
</tr>
<tr>
<td></td>
<td>Use of Omega 3 fatty Acids in Attenuating NAFLD ................................................................. 17</td>
</tr>
<tr>
<td>III</td>
<td>MATERIALS AND METHODS ................................................................. 25</td>
</tr>
<tr>
<td></td>
<td>Subjects ................................................................. 25</td>
</tr>
<tr>
<td></td>
<td>Diets ................................................................. 25</td>
</tr>
<tr>
<td></td>
<td>Reagents ................................................................. 26</td>
</tr>
<tr>
<td></td>
<td>Fexofenadine-diet Interaction Study ................................................................. 26</td>
</tr>
<tr>
<td></td>
<td>Body Weights ................................................................. 27</td>
</tr>
<tr>
<td></td>
<td>Blood Sample Collection ................................................................. 28</td>
</tr>
<tr>
<td></td>
<td>Analysis of Fasting Blood Glucose ................................................................. 28</td>
</tr>
<tr>
<td></td>
<td>Tissue Collection ................................................................. 28</td>
</tr>
<tr>
<td></td>
<td>Analysis of Serum Triglycerides ................................................................. 29</td>
</tr>
<tr>
<td></td>
<td>Data Analysis and Statistical Methods ................................................................. 29</td>
</tr>
<tr>
<td>IV</td>
<td>RESULTS ................................................................. 30</td>
</tr>
<tr>
<td></td>
<td>Effect of Diet Alone on Body Weight ................................................................. 30</td>
</tr>
<tr>
<td></td>
<td>Interactive Effects of Fexofenadine, Diet, and Time on Body Weight ................................................................. 31</td>
</tr>
<tr>
<td></td>
<td>Effect of Fexofenadine on Body Weight ................................................................. 31</td>
</tr>
<tr>
<td></td>
<td>Effects of Diet and Drug on Tissue and Organ Weights ................................................................. 38</td>
</tr>
<tr>
<td></td>
<td>Analysis of Fasting Blood Glucose ................................................................. 39</td>
</tr>
<tr>
<td></td>
<td>Analysis of Serum Triglycerides ................................................................. 40</td>
</tr>
<tr>
<td>V</td>
<td>DISCUSSION ................................................................. 41</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>................................................................. 46</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Distribution of Weight, BMI, Waist Circumference, and Insulin Concentration in Adult H1 Antihistamine Users and Matched Controls.</td>
</tr>
<tr>
<td>2</td>
<td>Factorial Experimental Design: Daily Administration of Fexofenadine 40 mg/kg/mouse or Vehicle Control: 10% Lard, 45% Lard, and 45% Omega-3 Fatty Acid Diets</td>
</tr>
<tr>
<td>3</td>
<td>Fat Pad Weights, Organ Weights, and Fasting Blood Glucose (FBG) Concentrations</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>Two-hit hypothesis indicating progression from NAFLD to NASH</td>
</tr>
<tr>
<td>2</td>
<td>Interrelationship between peripheral insulin resistance and hepatic fat deposition</td>
</tr>
<tr>
<td>3</td>
<td>Peripheral insulin resistance and fatty liver</td>
</tr>
<tr>
<td>4</td>
<td>The role of histamine and diet in metabolic disturbances</td>
</tr>
<tr>
<td>5</td>
<td>Potential triglyceride (TG)-lowering mechanisms of the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid</td>
</tr>
<tr>
<td>6</td>
<td>Effect of dietary fat (vehicle only) on body weight, study one</td>
</tr>
<tr>
<td>7</td>
<td>Effect of dietary fat (vehicle only) on body weight, study two</td>
</tr>
<tr>
<td>8</td>
<td>Effect of fexofenadine on 9 day weight gain/10% lard diet, study one</td>
</tr>
<tr>
<td>9</td>
<td>Effect of fexofenadine on 9 day weight gain/45% lard diet, study one</td>
</tr>
<tr>
<td>10</td>
<td>Effect of fexofenadine on 9 day weight gain/45% omega-3 diet, study one</td>
</tr>
<tr>
<td>11</td>
<td>Effect of fexofenadine on 9 day weight gain/10% lard diet, study two</td>
</tr>
<tr>
<td>12</td>
<td>Effect of fexofenadine on 9 day weight gain/45% lard diet, study two</td>
</tr>
<tr>
<td>13</td>
<td>Effect of fexofenadine on 9 day weight gain/45% omega-3 diet, study two</td>
</tr>
<tr>
<td>14</td>
<td>Effect of fexofenadine on 4 week weight gain in 10% lard diet, study two</td>
</tr>
</tbody>
</table>
15 Effect of fexofenadine on 4 week weight gain in 45% lard diet, study two ................................................................. 37

16 Effect of fexofenadine on 4 week weight gain in 45% omega-3 diet, study two ................................................................. 38
CHAPTER I
INTRODUCTION

Metabolic syndrome is identified as a set of risk factors that significantly increase an individual’s susceptibility toward heart disease, stroke, and diabetes. To confirm a diagnosis of metabolic syndrome, three or more of the following factors must be present: visceral adiposity (>40 inch waistline for men and >35 inches for women), hypertriglyceridemia (>150 mg/dl), elevated fasting blood glucose (FBG) (>100 mg/dl), hypertension (>130/85 mm Hg), and low high density lipoprotein (HDL) levels (<40 mg/dl for men and <50 mg/dl for women) (American Heart Association, n.d.). Epidemiological studies indicate that approximately 34% of American adults suffer from metabolic syndrome (Ford, Giles, Dietz, 2002). While the etiology of metabolic syndrome is not well understood, it is recognized to share many characteristics with insulin resistance and obesity. Nonalcoholic fatty liver disease (NAFLD) is a common co-morbidity of the disorder (Vanni et al., 2010). Although not characterized as a defining criterion of metabolic syndrome, NAFLD is considered to be the primary hepatic manifestation of metabolic syndrome (Vanni et al., 2010).

NAFLD, a major cause of global liver disease (Younossi et al., 2016), has a prevalence that is growing in pace with obesity (Bertot & Adams, 2016). It is a disease spectrum marked by hepatic lipid accumulation (simple hepatic steatosis) followed by hepatocyte injury, inflammation, and fibrosis (Xu, Ding, &
Qiao, 2010). The pathophysiological continuum of NAFLD extends from steatosis to nonalcoholic steatohepatitis (NASH) (Tarantino, Savastano, & Colao, 2010). NASH, the most aggressive form of the disease, can progress from fibrosis to cirrhosis and, ultimately, to hepatocellular carcinoma (HCC). While the prevalence of NAFLD in the general population is approximately 30% in Western countries, it climbs to approximately 66% in those with obesity. In individuals with type 2 diabetes, the occurrence of NAFLD rises to approximately 70% (Targher et al., 2007).

In addition to metabolic syndrome, key influences connected to the development of NAFLD include a high-fat diet, obesity, and side effects of certain medications. Chronic high fat diets induce pathogenic changes in body weight, epididymal fat weight, liver weight, hepatic fat accumulation, and elevated serum markers of liver injury such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Tarantino, Savastano, & Colao, 2010). Further research supports causality between long term high fat diet feeding and significantly increased hepatic fat accumulation and plasma ALT levels, as well as increased body weight gain and systemic insulin resistance compared to mice fed a control diet (Wang et al., 2010).

Obesity is strongly correlated to NAFLD (Pallayova & Taheri, 2014). Cellular changes brought about in the development of obesity trigger hepatic cellular signaling changes related to the promotion of fatty liver disease.
(Pallayova & Taheri, 2014). For example, one such cellular change concerns hepatic insulin signaling. Insulin resistance has become well-recognized as a key player in the development of steatosis and the transitional process to steatohepatitis in NAFLD (Qureshi & Abrams, 2007; Jou, Choi, & Diehl, 2008).

Many studies have shown a variety of classes of medications, both prescription and over-the-counter, to be correlated with weight gain and liver injury. For instance, histamine 1 receptor (H1R) antagonists, typically used to alleviate allergy symptoms, are a class of medications that include weight gain as a possible side effect (Kalucy, 1980). Epidemiological data have found a strong association of H1R antagonists with increased weights, waist circumferences, and insulin concentrations when compared to matched controls (Ratliff, Barber, Palmese, Reutenauer, & Tek, 2010). Additionally, the combination of H1 antihistamines with a high fat diet in mice has demonstrated an exacerbated state of hepatic steatosis (Raveendran et al., 2014).

Although there are currently no FDA-approved treatments for NAFLD or NASH, murine models suggest that dietary supplementation with omega 3 fatty acids offers a protective role against NAFLD progression (Oliveira et al., 2006). In fact, supplementation with omega 3 fatty acids has been found to prevent or reverse hepatic steatosis in mice (Oliveira et al., 2006). To date, no studies have evaluated the effects of omega 3 fatty acid supplementation on the prevention and/or progression of NAFLD induced by a high fat diet and H1
antihistamine administration. In addition, few studies have examined the interactive hepatotoxic effects of high or low fat diet combined with chronic H₁ antihistamine administration. Taken together, these factors indicate a gap in the research that provides the impetus for this current study.

**Objectives of Study**

In the present study, the primary objective has been to examine the effects of the selective H₁-antagonist fexofenadine in combination with different levels of dietary fats on weight gain, metabolic disturbances, and hepatic steatosis in mice. A secondary objective has been to evaluate the potential protective effects incurred via substitution of omega-3 fatty acids for lard in fexofenadine-induced weight gain and metabolic disruptions.

**Hypothesis**

Combined daily administration of fexofenadine and a high fat diet (45% of kilocalories from lard) will demonstrate significant increases in body weight, metabolic disturbances, and hepatic fat accumulation when compared to control (10% of kilocalories from lard). Moreover, daily administration of fexofenadine combined with an omega-3 diet (45% of kilocalories from menhaden oil) will result in body weights and hepatic fat levels that do not differ significantly from control.
 CHAPTER II
LITERATURE REVIEW

Incidences of metabolic disorders, such as obesity, insulin resistance, and metabolic syndrome, are steadily increasing in Westernized societies. Metabolic syndrome is a disorder characterized by visceral fat, hypertension, hyperglycemia, hypertriglyceridemia, and low HDL levels. A combination of three or more of these factors confirms a metabolic syndrome diagnosis and significantly increases the probability of cardiovascular events and diabetes. Due to the systemic nature of metabolic disturbances, numerous physiological functions are affected in patients with metabolic syndrome, obesity, and insulin resistance. In particular, the hepatic manifestation of metabolic syndrome is an accumulation of fat in the liver known as hepatic steatosis or nonalcoholic fatty liver disease (NAFLD) (Vanni et al., 2010).

As the occurrence of obesity has risen steeply over the past 30 years, the prevalence of NAFLD has followed suit. Currently, about 68% of adults in the United States are overweight or obese (Flegal, Carroll, Ogden, Curtin, 2010) and approximately 30% have NAFLD (Williams et al., 2011). Affecting approximately 70 million people in the United States, NAFLD is the most common chronic liver disease in America (Alkhouri & McCullough, 2010). Characterized by triglyceride accumulation in hepatocytic cytoplasm, NAFLD is defined as 5 – 10% hepatic fat accumulation in non-alcoholic individuals (<20 g ethanol/d) and in whom other
known causes of steatosis have been excluded (McCullough, 2004).

The spectrum of NAFLD begins with simple steatosis and progresses to nonalcoholic steatohepatitis (NASH) followed by fibrosis and advanced liver disease. The “two-hit hypothesis” of NASH describes progression of the disease beginning with an accumulation of hepatic lipids (hit one), which weaken the liver, making it susceptible to subsequent oxidative, hepatotoxic events (hit 2) (Figure 1) (Sharma et al., 2015). Therefore, while simple steatosis is relatively benign, it potentially sets the stage for the development of NASH. NASH is characterized by hepatic injury including hepatocyte ballooning and cell death, increased blood levels of the hepatic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), hepatic inflammation, oxidative stress, and fibrosis (Farrell & Larter, 2006; Cohen, Horton, & Hobbs, 2011; Hashimoto, Tokushige, & Farrell, 2012). Associated with numerous adverse clinical outcomes, NASH has been shown to contribute to cirrhosis, hepatocellular carcinoma, and liver-related death (Zivkovic, German, & Sanyal, 2007).

While the mechanisms underlying the development and progression of NAFLD are yet to be fully understood, insulin resistance and obesity-related inflammation demonstrate a strong correlation to hepatic steatosis (Choudhury & Sanyal, 2004). Correspondingly, the influence of diet on metabolism and metabolic regulation - through its effects on hormones, transcription factors, and lipid metabolic pathways - has proven to be a fundamental contributor in
the etiopathogenesis of NAFLD (Choudhury & Sanyal, 2004). With diet also holding a key role in the development of insulin resistance and obesity, it emerges as a crucial piece of the NAFLD/metabolic syndrome/obesity puzzle.

![Diagram](image)

**Figure 1.** Two-hit hypothesis indicating progression from NAFLD to NASH. Illustration of known and unknown factors involved in nonalcoholic liver disease progression. Adapted from Sharma et al., 2015.

To date, there are no FDA-approved treatments for NAFLD or NASH. With diet being implicated in the development of NAFLD, dietary modifications are cornerstone in the prevention and reversal of the disease. In particular, omega-3 fatty acids have demonstrated both prevention and reversal of hepatic steatosis in mice (Oliveira et al., 2006). In addition, omega-3 fatty acids have shown a reduction in hepatic fat in humans (Parker et al., 2012). Beneficial outcomes related to inflammation and metabolism are two key mechanisms modulated by omega-3 fatty acids. No research to date, however, has evaluated the effects of omega 3 fatty acids on the prevention and/or progression of high fat diet and H1 antihistamine-induced NAFLD.
The Role of Diet in NAFLD

Based on numerous animal models, extensive support exists in the literature for the role of diet in the etiology of NAFLD. In particular, a high-fat diet has been directly correlated to the development of steatosis. Using a long-term high-fat diet model, Wang et al. demonstrated a significant increase in hepatic fat accumulation and elevated plasma ALT levels in mice. In addition, weight gain and systemic insulin resistance were significantly increased when compared to control (Pallayova & Taheri, 2014).

Xu, Fan, Ding, and Qiao (2010) determined that a high fat diet accurately modeled the etiopathogenesis of NAFLD and its progression to NASH. Observing Sprague-Dawley rats, the researchers demonstrated high fat diet-induced pathogenic changes in body weight, liver weight, perigonadal fat weight as well as elevations in serum markers of NAFLD including ALT, free fatty acids, insulin, and tumor necrosis factor-alpha (TNF-alpha). Liver histology revealed progressive steatosis, inflammation, and perisinusoidal fibrosis with an exacerbation of symptoms beginning at week 4 and continuing through week 48 of the study.

Employing a long-term high fat feeding model, Nakamura and Terauchi (2013) induced NASH progression and liver tumorigenesis in C57Bl/6J male mice. Specifically, mice fed a high fat diet developed significantly higher body
weights, liver weights, fasting insulin levels and leptin levels along with significantly lower adiponectin levels compared to control.

In addition to diet, obesity plays a key role in the development of NAFLD. While the causal relationship between obesity and NAFLD needs further elucidation, it is clear that cellular changes brought about in the development of obesity trigger cellular signaling disruptions responsible for promoting fatty liver disease. One particular pathway, insulin resistance, has been recognized as a cellular signaling pathway gone awry in the development of steatosis and in the transitional progression from steatosis to steatohepatitis (Hudgins et al., 2000; Girman et al., 2004; Kim et al., 2004). In fact, researchers have successfully ameliorated NAFLD via attenuation of insulin resistance. In particular, pharmaceutical insulin sensitizers, thiazolidinediones (TZDs) and metformin, have shown improvements in the prevention and/or attenuation of NAFLD (Bugianesi et al., 2005; Parks, 2002; Choudhury & Sanyal, 2004). Unfortunately, common side effects such as weight gain and increased body fat have impeded their long-term usefulness as therapeutic agents (Sanyal et al., 2001; Charlton, Sreekumar, Rasmussen, Lindor, & Nair, 2002).

While there is wide support for the role of insulin resistance in the development of NAFLD, there is a lack of consensus over the initial site of NAFLD-related insulin resistance – whether it is peripheral or hepatic. The majority of research, however, supports the concept that insulin resistance
originates in peripheral adipose tissue (Marchesini et al., 2003; Ong et al., 2005). Once peripheral insulin resistance is established, hepatic insulin resistance is thought to follow, resulting in further exacerbation of overall systemic insulin resistance (Birkenfeld & Shulman, 2014). Hepatic insulin signaling is therefore thought to be a secondary disruption in cellular signaling that contributes to NAFLD (Birkenfeld & Shulman, 2014).

In normal functioning, the release of insulin suppresses lipase which, in turn, inhibits lipolysis - the catabolism and release of free fatty acids (FFA) from adipose tissue. As peripheral insulin resistance develops and insulin concentrations continue to rise, however, suppression of lipolysis lags behind, resulting in increased FFAs in the portal circulation (Figure 2). As a result, the increased hepatic exposure and uptake of FFA result, not only in disproportionate hepatocyte fat accumulation, but also in hepatic insulin resistance (McCullough, 2004).

![Figure 2](image-url). Interrelationship between peripheral insulin resistance and hepatic fat deposition. Excessive caloric intake induces lipolysis, tumor necrosis factor-α (TNF-α) expression and reduced adiponectin levels, leading to peripheral insulin resistance and increased circulating fatty acids. Hepatic fat deposition induces insulin resistance through disturbed intracellular insulin signaling (Mendez-Sanchez, Arrese, Zamor-Valdes, & Uribe, 2007).
Also in regular functioning, post-prandial insulin release inhibits hepatic glucose synthesis through the suppression of gluconeogenesis and glycogenolysis while at the same time stimulating glycogen synthesis and lipogenesis (Farese, Zechner, Newgard, & Walther, 2012). By regulating glucose and lipid metabolism, hepatic insulin signaling helps to maintain energy homeostasis. In hepatic insulin resistance, however, insulin secretion fails to suppress hepatic glucose production. Interestingly, while hepatic steatosis disrupts glucose regulation, it leaves de novo lipogenesis unchanged, resulting in continued synthesis and accumulation of hepatic triglycerides (Nakamura & Terauchi, 2013). One explanation of this phenomenon is called “selective insulin resistance”, whereby disturbed insulin signaling pathways disrupt glucose metabolism and adipocytic lipolysis but leave hepatic lipogenesis intact, contributing to the co-existence of hyperglycemia and dyslipidemia in insulin-resistant states (Figure 3) (Zivkovic, German, & Sanyal, 2007; Brown & Goldstein, 2008).
Figure 3. Peripheral insulin resistance and fatty liver. While multiple mechanisms are addressed in this figure, key components pertinent to this study include: accelerated adipocyte lipolysis resulting from insulin resistance results in the influx of plasma free fatty acids from adipose tissue to the liver. This represents the predominant source of intrahepatic triglycerides. De novo lipogenesis, which accounts for less than 5% in healthy subjects, increases to 25% in NAFLD patients contributing a significant secondary source of hepatic triglycerides.\textsuperscript{48} Chylo = chylomicron. Adapted from Zivkovic, German, & Sanyal (2007).

In keeping with these data, Donnelly et al. determined that in NAFLD patients, adipose-released free fatty acids (FFAs) in the plasma were the primary contributor to hepatic triglyceride content (50–70% of total fatty acids). In the fasted state, de novo lipogenesis was upregulated, accounting for 25% of liver triglycerides compared with 5% in healthy individuals (Donnelly et al., 2005). Also de novo synthesis of fatty acids from glucose, fructose, and amino acids has been shown to be impaired in NAFLD patients (Hudgins et al., 2000). Consequently, in peripheral insulin resistance, adipose-induced fatty acids remain elevated and enhanced rates of lipogenesis significantly contribute to hepatic triglyceride accumulation (Hudgins et al., 2000).
**H₁ Antihistamine Use and Metabolic Disturbances**

In addition to diet, histamine dysregulation has been shown to play a part in metabolic disturbances including the development of NAFLD. Histamine, a neurotransmitter released by the posterior hypothalamus, mediates numerous vascular and immunological functions such as vasodilation and local immune response (Benly, 2015). Additionally, histamine regulates allergy-related inflammation, energy intake, energy expenditure, and body-weight homeostasis (Raveendran et al., 2014). Histamine receptors are divided into four different types: histamine-1 receptor (H₁R), H₂R, H₃R, and H₄R. Histamine₁R and H₃R are profusely expressed in the brain and linked to appetite regulation (Haas, Sergeeva, & Slebach, 2008).

Decades of animal research have validated histamine’s role in energy homeostasis. Intravascular administration of histamine in cats has demonstrated a reduction in food intake (Clineschmidt & Lotti, 1973). Mice infused with histamine have exhibited lowered adiposity and fat accumulation (Masaki, Yoshimatsu, Chiba, Watanabe, & Sakata, 2001). Histamine antagonism has induced increased food consumption in rats (Sakata, Yoshimatsu, & Kurokawa, 1997). Additionally, in humans, an epidemiological cross-sectional analysis has established that adults taking prescription H₁R blockers experience significant increases in weight, waist circumference, and insulin concentration when compared to matched controls (Table 1) (Ratliff, Barber, Palmese,
Recent reports using histamine receptor knockout mice along with histadine decarboxylase (HDC) knockout mice have provided insight into histamine’s role in metabolism and weight regulation. HDC, the rate-limiting enzyme involved in histamine synthesis, and H₁R knockout mouse models have demonstrated glucose intolerance and abdominal adiposity, suggesting that histamine signaling via histamine receptors is a key factor in obesity regulation (Fülöp et al., 2003; Masaki, Yoshimatsu, Chiba, Watanabe, & Sakata, 2001). A study by Fülöp et al. (2003) using HDC knockout mice demonstrated clinical characteristics of visceral adiposity, hyperleptinemia, and decreased glucose tolerance. Additionally, Masaki, Yoshimatsu, Chiba, Watanabe, & Sakata (2001)
found that H₁R knockout mice fed a high-fat diet showed increased fat deposition and leptin resistance. Furthermore, Wang et al. (2010) found H₁R knockout mice developed an obese phenotype with visceral adiposity, hyperleptinemia, hepatic steatosis and inflammation with increased hepatic triglyceride. These data support the critical nature of H₁R signaling in glucose regulation and lipid metabolism as well as in the development of hyperlipidemia-induced NAFLD.

In addition to weight-related metabolic disturbances, H₁-antihistamines have been shown to play a significant role in the exacerbation of NAFLD. In examining the effects of H₁-antihistamines on the progression of diet-induced fatty liver disease, Raveendran et al. (2014) concluded that routine use of H₁-antihistamines induced increases in body weight gain, liver weight, gonadal fat deposition, biomarkers of liver injury, and hepatic steatosis in high fat diet-fed wild type mice. In addition, they determined that chronic administration of fexofenadine - a commonly used H₁-antihistamine - increased serum glucose levels, as well as hepatic triglyceride and cholesterol ester levels.

In a study aimed at understanding the mechanistic relationship between H₁-antagonist antipsychotics and weight gain, Liu, Lian, Hu, and Deng (2015) examined the effects of an indirect H₁ agonist (betahistine) on olanzapine-induced dyslipidemia. Olanzapine is one of the most potent H₁ antagonists in the realm of antipsychotics. When the two were co-administered, the
researchers discovered a reversal of olanzapine-induced dyslipidemia. In addition, they demonstrated that the effects were based upon betahistine’s activation of hepatic adenosine monophosphate-activated protein kinase α (AMPK α) coinciding with a significant reduction in nuclear sterol regulatory element-binding protein-1 (SREBP-1) protein expression and increased expression of peroxisome proliferator-activated receptor-α (PPARα). These results confirm prior data where an inverse correlation was determined between AMPK and SREBP-1 in hepatocytes and mouse livers (Awazawa et al., 2009). Liu and colleagues’ research supports the ability of H1R agonists to act upon hepatic H1 receptors via modulation of AMPKα, SREBP-1 and PPARα-dependent pathways to improve drug-induced dyslipidemia.

From a mechanistic standpoint, He et al. (2009) determined that histamine increases hepatic triglyceride lipase (HTGL) levels and HTGL mRNA expression. Given that HTGL is the rate-limiting enzyme involved in triglyceride hydrolysis and that it regulates the uptake of fatty acids into tissues, histamine stimulates lipid metabolism by its effects on HTGL and on the upregulation of HTGL mRNA expression. Therefore, the use of H1 antagonists potentially decreases HTGL activity resulting in a reduction of hepatic triglyceride lipolysis with an ensuing buildup of triglycerides in the liver.

In summary, the interactive effects of a high fat diet and chronic H1 antihistamine administration impact multiple mechanisms -- from insulin
resistance to HTGL suppression -- that promote systemic metabolic disruptions including hepatic fat accumulation (Figure 4).

**Histamine, Diet and Hepatocytes**

*Figure 4. The role of histamine and diet in metabolic disturbances. Illustration of numerous cascades involved in disrupted metabolic pathways.*

**Use of Omega 3 Fatty Acids in Attenuating NAFLD**

While there are currently no FDA-approved treatments for NAFLD, emerging research supports the role of nutrition in developing safe and therapeutic approaches to both prevention and treatment (Papandreou & Andreou, 2015; Fan & Cao, 2013; Freidoony & Kong, 2014). Interestingly, decades of research have supported a correlation between disproportionate essential fatty acid (EFA) ratios and steatosis (Werner et al., 2005; Alwayn et al., 2004; Bouziane, Prost, & Belleville, 1994). Although Western diets are energy rich, it is widely recognized that they provide significantly incongruent EFA ratios.
EFAs -- omega 3 fatty acids and omega 6 fatty acids -- are a class of lipids known as polyunsaturated fatty acids (PUFAs) that the human body is incapable of synthesizing. As a result, it is essential to obtain EFAs from the diet. Both omega-3 and -6 fatty acids are vital components of practically all cell membranes (Linus Pauling Institute, n.d.). Although omega-6 fatty acids are abundant in processed foods and vegetable oils, omega-3 fatty acids are significantly lacking in Western diets resulting in deficient omega-3 intakes. The proper ratio of omega-6 to omega-3 fatty acids is necessary for healthful development throughout the human life cycle, including functional development of the brain, retina, and spermatozoa (Simopoulos, 2011). While research supports an ideal human ratio of omega-6 to omega-3 ranging from 4:1 to 1:1, Western diets typically comprise an omega-6 to omega-3 dietary ratio of 25:1 to 10:1 (Lombardo & Chicco, 2006).

In addition to facilitating normal human development, adequate levels of omega-3 fatty acids are crucial for the following physiological functions: cell membrane morphology and function, effects on membrane receptors, provision of raw material for blood clotting hormones, contraction and relaxation of artery walls, antioxidant properties, and key roles in overall metabolism (Lombardo & Chicco, 2006). By interacting with specific transcription factors, such as PPARs, they also serve to regulate particular genetic functions including those involved with fatty acid metabolism and inflammation (Martin, 2010). Omega 3-derived
long chain fatty acids comprise 40% of brain membrane phospholipid fatty acids and are involved in the synthesis and metabolism of neurotransmitters (Knochel et al., 2015). By facilitating the repair and regeneration of oxidative stress-induced cell membrane damage, omega 3 fatty acids mitigate liver toxicity at both the biochemical and cellular level. (Pinel, Morio-Liondore, & Capel, 2014). In addition, omega 3 fatty acids have been shown to offer immunomodulatory, antidiabetic, and cardioprotective benefits (Chavan et al., 2013).

Rich sources of omega-3 fatty acids include fatty fish, flaxseeds, walnuts, and chia seeds. The protective effects of these oils are thought to originate directly from their components, alpha linolenic acid (ALA) and the long chain polyunsaturated fatty acids -- eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are found solely in marine-based sources of omega-3s such as salmon and cod liver oil. ALA, a plant-based omega-3 fatty acid derivative, is found predominantly in flaxseeds, walnuts, and chia seeds. Although ALA can be converted to DHA and EPA in the human body, the conversion is quite limited and inefficient (Gerster, 1998). Therefore, in order to obtain adequate intakes of EPA and DHA, it is critical to consume or supplement adequate amounts of marine-derived omega 3 fatty acids.

The effects of omega-3 fatty acids on dyslipidemia and insulin resistance have been extensively researched (Lombardo & Chicco, 2006; Lalia & Lanza, 2016; Pinel, Morio-Liondore, & Capel, 2014). Supplemental DHA and EPA
demonstrate impressive results in animal models of insulin resistance with benefits such as: 1) lowered plasma triglycerides, free fatty acids, glucose, and insulin, 2) peripheral insulin resistance prevention, 3) reduced hepatic triglycerides, VLDLs, and lipogenesis, 4) lowered lipid concentrations, 5) improved skeletal muscle glucose utilization and storage, 6) decreased adipocyte cell size and visceral fat content, and 7) increased insulin-stimulated glucose transport in the adipose tissue (Depner et al., 2013).

Further results from animal models provide persuasive evidence that omega-3 fatty acids are a key dietary supplement for patients with NAFLD. Depner et al. (2013) found that an accumulation of various hepatic lipids including omega-6 fatty acids, combined with an omega-3 fatty acid depletion, play a major role in NASH-associated pathologies, including hepatosteatosis, inflammation, oxidative stress and fibrosis in low density lipoprotein receptor (LDLR) knockout mice. In a study observing the impact of omega 3 fatty acids on hepatic triglycerides, Levy, Clore, and Stevens (2004) showed that fish-oil fed animals experienced 27% (p< 0.05) and 73% (p< 0.01) reductions in hepatic triglycerides vs. control- and lard-fed animals, respectively. Fish oil modified postprandial gene expression of hepatic mechanisms of fatty acid metabolism. Specifically, supplementation with fish oil inhibited the normal postprandial decline in fatty acid catabolism genes (PPARα, CPT1, and ACO) and curtailed the normal postprandial increase in triglyceride synthesis genes (SREBP1-c, FAS,
SCD-1). Consequently, DHA and EPA promoted the catabolism of fatty acids via the activation of PPAR–mediated pathways along with the down-regulation of de novo lipogenesis through SREBP pathways. Accordingly, the researchers determined that omega-3 fatty acid supplementation directly decreased postprandial hepatic triglyceride storage as well as total body weight, total body fat, and hepatic steatosis (Levy, Clore, & Stevens, 2004).

The molecular mechanisms involved in serum triglyceride reductions via omega-3 fatty acids are not fully known, but potential mechanisms derived from preclinical studies are illustrated in Figure 5. Based on these studies, omega-3 fatty acids reduce hepatic triglyceride synthesis and secretion and decrease free fatty acid delivery from adipocytes (Harris & Bulchandani, 2006; Davidson, 2006; Wassall & Stillwell., 2008).
Figure 5. Potential triglyceride (TG)-lowering mechanisms of the long chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. While many mechanisms are addressed in this figure, those most pertinent to this study include: Decreased de novo lipogenesis results from down-regulation of sterol regulatory element-binding proteins (SREBP)-1c (1), leading to reduced substrate for TG synthesis and decreased activity of TG-synthesizing enzymes (5). In addition, decreased free fatty acid delivery from adipose tissue is related to activation of PPAR gamma (6). All comprise potential mechanisms for reduced TG accumulation in the liver. Adapted from Harris & Bulchandani (2006), and Davidson (2006).

In examining the effects of omega-3 fatty acid supplementation in patients with metabolic disturbances, Campanni and colleagues (2006) demonstrated that NAFLD patients taking 1 g fish oil/day for 12 months decreased blood triglyceride concentrations, liver enzymes, fasting glucose, and steatosis. In patients taking 2 g of fish oil/day for six months, Spadaro et al. (2006) found a reduction in blood triglycerides, liver enzymes, TNF-α as well as regression of steatosis. When type 2 diabetic patients supplemented ALA by consuming 30 g walnuts/day for eight weeks, they experienced a decrease in plasma low-density lipoproteins (LDLs), an increase in plasma high-density lipoproteins (HDLs), and an increase in the ratio of HDL cholesterol to total...
cholesterol (TC) (Tapsell et al., 2004). Another study showed that patients with high cholesterol who substituted 32% of their monounsaturated fatty acid intake with walnuts experienced significant decreases in TC and LDLs along with improved endothelial function compared with controls (Ros et al., 2004). Results of these studies and others provide convincing evidence that omega-3 supplementation from fish oils and plant-based sources improves blood lipid profiles while reducing inflammation, steatosis, and liver damage in NAFLD patients.

Two of the most important characteristics of omega-3 hepatoprotection include antioxidant and anti-inflammatory activities. A study by Meganathan et al. (2011) indicates that omega-3 fatty acids protect the liver against paracetamol-induced liver injury. Although the exact mechanism of hepatoprotection by omega-3 fatty acids is not completely understood, omega-3s are thought to diminish toxin-induced hepatocyte damage via their function as antioxidants/cofactors. Additional proposed protective mechanisms include improved hepatocyte membrane stabilization and conversion of omega-3 fatty acids to lipid protective mediators (Meganathan et al., 2011). EPA and DHA also attenuate hepatic inflammation, at least in part, by inhibiting the cellular response to the inflammatory stimuli such as cytokines and plasma endotoxin and by decreasing the ratio of omega-6 fatty acids to omega-3 fatty acids in membrane phospholipids. This shift in membrane makeup has also been shown
to disrupt lipid rafts (Wassall & Stillwell, 2008) and suppress TLR4 and nuclear NFκB content in omega-3 PUFA fed mice (Depner, Torres-Gonzalez, Tripathy, Milne, & Jump, 2012).

The goals of this study were to evaluate the effects of fexofenadine and a high fat diet on metabolic disturbances and to assess the role of omega-3 fatty acids in the attenuation of such disturbances. In the present experiment, we examined whether chronic administration of fexofenadine at therapeutic doses along with a high fat diet would promote body weight gain and increased fat pad weights. Moreover, we assessed the impact of a high omega-3 fat diet on weight gain, fat pad mass, kidney and liver weights.
CHAPTER III
MATERIALS AND METHODS

Subjects

Study one included 48 and study two included 72 male C57Bl/6J mice (Mus musculus), obtained from a breeding colony at Indiana University of Pennsylvania (IUP). Founders of the colony originally came from Jackson Laboratories (Bar Harbor, Maine, USA). At the start of the experiment, the mice weighed approximately 25 grams and were 8-12 weeks old. Mice were maintained in the animal facility at IUP on a 12:12 light:dark cycle at approximately 22 to 25° C. All subjects had free access to food and water. Only animals that completed the study were included in the full analysis. The experiment was carried out in compliance with policies of the IUP Institutional Animal Care and Use Committee (IACUC).

Diets

Study one used a run-in period on one to two weeks and study two used a run-in period of two days. Mice were maintained on LabDiet 5P00/RMH 3000 (T.R. Last, Cabot, PA). After initiation of the experiment, the mice were fed ad libitum one of three diets: a high fat diet (45% of dietary calories from lard), an omega-3 diet (homologous to the high fat diet with menhaden oil substituted for lard), or control diet (10% dietary calories from lard) (Research Diets D12450J, D12451, and D03022403, respectively). The sucrose content of these diets was
constant (691 kcal) across all diets. Corn starch caloric content was 2025, 291 and 291 kcal for the 10% fat, 45% fat, 45% omega-3 diets, respectively.

**Reagents**

Fexofenadine was obtained from AK Scientific, Inc. (California) and was dissolved in distilled water. The pH was adjusted to ~5 using NaOH. Dosing of 40 mg/kg was determined based on the daily maximum therapeutic doses for human adults back-translated for mice\(^\text{16}\). Vehicle was phosphate buffered saline. Drug or vehicle was administered once daily (q.d.) intraperitoneally (i.p.).

**Fexofenadine-diet Interaction Study**

To allow animals to get acclimated to handling and injections, a run-in period of one to two weeks or two days was administered (study one and study two, respectively). Mice were randomly assigned into one of six different drug-diet treatment groups (described below) with each group containing twelve mice (see Table 2). The study ran for nine days and four weeks (study one and study two, respectively). No significant differences in initial body weights existed between treatment groups. The negative control group received saline injections. The experimental group received fexofenadine at 40 mg/kg body weight. Mice were weighed each morning prior to treatment in order to determine proper injection volumes. All injections were administered i.p. Injections were given q.d. each morning.
Mice were fed ad libitum and randomly assigned to one of three diets. The three diets were differentiated by type of fat and amount of calories derived from fat including: a high fat diet with 45% of calories derived from lard, an omega-3 fatty acid diet with 45% of calories derived from menhaden oil, a low fat diet with 10% of calories derived from lard. Diet was weighed and renewed approximately every three to five days. Twelve hours prior to euthanization, all food was discontinued in order to determine fasting blood glucose levels. All animals were sacrificed via cervical dislocation at the end of the study.

Table 2
Factorial Experimental Design: Daily Administration of Fexofenadine 40 mg/kg/mouse or Vehicle Control; Diets: 10% Lard, 45% Lard, and 45% Omega-3 Fatty Acid Diets.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>10% Lard</th>
<th>45% Lard</th>
<th>45% Omega3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fexofenadine 40 mg/kg</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Body Weights

Baseline body weights were determined one day prior to the onset of diet and drug administration. Body weights were measured and recorded daily with the final weights being taken immediately prior to euthanization. Body weights
were transformed into percent control baseline body weights by dividing a given
daily body weight by the baseline body weight and multiplying by 100.

**Blood Sample Collection**

Prior to euthanization, approximately 0.3 uL of blood was taken by tail
nick to determine fasting blood glucose (FBG) and approximately 100 µL of blood
was taken from each animal by milking blood from the tail nick. Blood collections were
done with Microvette® tubes from Sarstedt (Nümbrecht, Germany). The blood was kept
on ice until it was centrifuged for 6 minutes at 10,000x G at 4 °C. Serum was then stored
at -80 °C for triglyceride assay analyses.

**Analysis of Fasting Blood Glucose**

Fasting blood glucose concentrations were measured using a ReliOn
glucose meter and test strips.

**Tissue Collection**

Following euthanasia, mouse livers and kidneys were removed and
weighed. Perirenal, perigonadal, and retroperitoneal fat pads were also removed
and weighed. The kidneys and partial liver sections from each mouse were then
fixed in 10% neutral buffered formalin and preserved for future
histopathological assessment. The remaining liver sections were frozen and
stored in -80° for hepatic triglyceride assay analyses.
**Analysis of Serum Triglycerides**

For serum triglyceride analysis, a triglyceride colorimetric kit from Cayman Chemical (Cat No. 10010303) (Ann Arbor, MI) was used. After blood sample collection, the blood was kept on ice until it was centrifuged for 6 minutes at 10,000× G at 4°C. Serum samples were then stored at -80°C. Samples were analyzed colorimetrically following the manufacturer’s protocol.

**Data Analysis and Statistical Methods**

Body weight data were analyzed by repeated measures analysis of variance (RM-ANOVA) using SYSTAT Statistical Software (SYSTAT Software, Inc., San Jose, CA). Drug and diet were between groups factors, while time was a within groups factor. The vehicle (PBS) and 10% fat diet were considered controls for these studies. Three-way ANOVAs with repeated measures analyses evaluated the between groups factors (drug and diet) along with the within groups factor (time). Due to significant interactions found between drug and time, follow up two-way ANOVAs and contrast analyses were run to assess the effects of drug over time within each diet. For measurements with only one time point (e.g., serum triglycerides, food consumption, organ weights), standard two-way or one-way ANOVAs were used. Serum triglyceride values were log transformed to stabilize variance for ANOVA.
CHAPTER IV

RESULTS

Effect of Diet Alone on Body Weight

One objective of this study was to evaluate whether or not we observed diet-induced obesity in male C57Bl/6J mice fed one of three different diets (10% lard, 45% lard, 45% omega-3 fatty acid) and administered vehicle alone. In neither study one nor study two, were significant differences in average body weight (aBW) observed in any of the diets (p > 0.05) in vehicle-treated mice, during either study period (Figure 6A and 6B).

Figure 6. Effect of dietary fat (vehicle only) on body weight, study one. This figure illustrates results for 10% lard, 45% lard, and 45% omega-3 diets. No significant differences were found in body weights based on dietary fat, p > 0.05.
Interactive Effects of Fexofenadine, Diet, and Time on Body Weight

In study one, examination of the data revealed an increase in body weight for 45% lard fed mice across the nine days of the study. Other diet groups did not present body weight increases. Statistical analysis (three-way ANOVA) revealed significant main effects of diet ($F=7.028$, df=2,321, $p=0.005$) and time ($F=12.892$, df=8,168, $p<0.001$), but not of drug ($p>0.05$). In addition, a significant interactive effect of diet and drug was observed ($F=5.618$, df=2,21, p=0.045).

Effect of Fexofenadine on Body Weight

In study one, examination of the data revealed an interactive effect for fexofenadine and diet on body weight ($F=3.618$, df=2,21, p=0.045). Analysis of 10% lard diet: Using a two-way ANOVA, it was determined that mice maintained on the 10% fat diet and treated with fexofenadine did not have significantly
different average body weights (aBW) than vehicle-treated mice during any day of the study (p>0.05) (Figure 7A). **Analysis of 45% lard diet:** Mice maintained on the 45% lard diet and treated with fexofenadine revealed significant body weight increases attributable to main effects of drug (F=7.584, df=1,9, P=0.022) and day (F=14.609, df=8,72, p<0.001) and an interaction of day and drug (F=2.410, df=8,72, P=0.023) using a two-way ANOVA. Follow up contrast analysis indicated an interactive effect of drug and time for five of nine days of the study (p<0.05) (Figure 7B). **Analysis of 45% omega-3 diet:** For mice in the 45% omega-3 fat dietary group, aBW of fexofenadine-treated mice were not significantly different than vehicle-treated mice during any day of the study (p>0.05) (Figure 7C).

![10% Lard Diet](image)

*Figure 8.* Effect of fexofenadine on 9 day weight gain/10% lard diet, study one. Long run-in period (one - two weeks). No body weight gain in drug group with 10% lard diet, p>0.05.
Figure 9. Effect of fexofenadine on 9 day weight gain/45% lard diet, study one: Long run-in period (one - two weeks). Body weight gain in drug group with 45% lard diet, p<0.05.

Figure 10. Effect of fexofenadine on 9 day weight gain/45% omega-3 diet, study one: Long run-in period (one - two weeks). No body weight gain in drug group with 45% omega-3 diet, p>0.05.

In study two, examination of the nine-day data (three-way ANOVA) revealed significant main effects of diet (F=6.338, df=2,62, p=0.003), drug (F=19.776, df=1,62, p<0.001), and time (F=14.392, df=8,496, p<0.001). No interactive effect of diet and drug was observed (p>0.05).
Analysis of 10% lard diet: Using a two-way ANOVA, it was determined that mice maintained on the 10% fat diet experienced no effect of drug (p>0.05) but an effect of day (F=12.657, df=8,152, p<0.001) (Figure 8A). No day by drug interaction was observed. Analysis of 45% lard diet: Mice maintained on the 45% lard diet and treated with fexofenadine revealed a significant main effect of drug (F=10.632, df=1,22, P=0.004) and day (F=7.468, df=8,176, p<0.001) and an interaction of day and drug (F=2.004, df=8,176, P=0.048) using a two-way ANOVA (Figure 8B). Follow up contrast analysis indicated significant reduction in the 45% lard/fexofenadine group every day after day one (p<0.05). Analysis of 45% omega-3 diet: For mice in the 45% omega-3 fat dietary group, a two-way ANOVA revealed that aBWs of fexofenadine-treated mice were significantly reduced compared to vehicle-treated mice (F=11.478, df=1,21, P=0.003) and a main effect of day was determined (F=2.475, df=8,168, P=0.015) (Figure 8C). No significant day:drug interaction was observed (p>0.05). Contrast analysis showed significant reductions from day four through day nine (p<0.05).
Figure 11. Effect of fexofenadine on 9 day weight gain/10% lard diet, study two: Short run-in period (two days). No body weight gain/loss in drug group with 10% lard diet, p>0.05.

Figure 12. Effect of fexofenadine on 9 day weight gain/45% lard diet, study two: Short run-in period (two days). Body weight loss in drug group with 45% lard diet, p<0.05.
Figure 13. Effect of fexofenadine on 9 day weight gain/45% omega-3 diet, study two: Short run-in period (two days). Body weight loss in drug group with 45% omega-3 diet, p<0.05.

In study two, examination of the four-week data revealed an initial fexofenadine-related weight loss in 45% lard & omega-3 fed mice that appeared to be transient, p<0.05. While body weights dropped initially in the 45% lard & omega-3 diets, by week four they did not differ from control (p>0.05) (Figures 9A, 9B, and 9C).
Figure 14. Effect of fexofenadine on 4 week weight gain in 10% lard diet, study two: Short run-in period (two days). No body weight gain/loss in drug group with 10% lard diet, p>0.05.

Figure 15. Effect of fexofenadine on 4 week weight gain in 45% lard diet, study two: Short run-in period (two days). Initial body weight loss in drug group with 45% lard diet, p<0.05, followed by resolution of baseline body weight by week 4, p>0.05.
**Figure 16.** Effect of fexofenadine on 4 week weight gain in 45% omega-3 diet, study two: Short run-in period (two days). Initial body weight loss in drug group with 45% omega-3 diet, p<0.05, followed by resolution of baseline body weight by week 4, p>0.05.

**Effects of Diet on Tissue and Organ Weights**

At study two’s completion, fat pads (perigonadal, perirenal, retroperitoneal, and perirenal/retroperitoneal combined) were removed and weighed (Table 3). Diet was found to significantly increase all fat pad weights in the 45% lard and 45% omega-3 diets compared to 10% lard diet. Due to this significance, follow up one-way ANOVAs and Tukey post-hoc comparisons were run to evaluate the effects of 45% lard and 45% omega-3 diets on each type of fat pad weight; outcomes revealed a significant effect of diet on perigonadal (F=13.616, df=2,69, p<0.001), perirenal (F=8.296, df=2,68, p=0.001), retroperitoneal (F=5.714, df=2,69, p=0.005), and perirenal/retroperitoneal
combined weights ($F=15.8$, $df=2.68$, $p<0.001$). Neither a main effect of drug nor an interactive effect of diet and drug had an effect on any fat pad weights.

During dissection, livers and kidneys were removed and weighed. A two-way ANOVA revealed no main effect of diet, drug, nor a diet:drug interaction on either liver or kidney weights ($p>0.05$).

Table 3
Fat Pad Weights, Organ Weights, and Fasting Blood Glucose (FBG) Concentrations. Outcomes Represent 30 Days of Exposure to Fexofenadine 40mg/Kg I.P. Q.D. or Vehicle with Specified Dietary Fat Contents (10%, 45%, Or 45% Omega-3 Fat Diets). Results are Shown as Average and SEM for $N=12$ Mice per Drug-Diet Treatment Group.

<table>
<thead>
<tr>
<th>Perirenal</th>
<th>Retroperitoneal</th>
<th>Perigonadal</th>
<th>Liver</th>
<th>Kidney</th>
<th>FBG</th>
<th>Diet</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07</td>
<td>0.06</td>
<td>0.30</td>
<td>1.19</td>
<td>0.33</td>
<td>113</td>
<td>Ave</td>
<td>veh</td>
</tr>
<tr>
<td>0.02</td>
<td>0.01</td>
<td>0.05</td>
<td>0.10</td>
<td>0.02</td>
<td>5.69</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>0.07</td>
<td>0.03</td>
<td>0.35</td>
<td>1.31</td>
<td>0.35</td>
<td>119</td>
<td>Ave</td>
<td>fex</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>0.02</td>
<td>7.34</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>0.09</td>
<td>0.07</td>
<td>0.55</td>
<td>1.26</td>
<td>0.34</td>
<td>125</td>
<td>Ave</td>
<td>veh</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
<td>6.64</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>0.09</td>
<td>0.07</td>
<td>0.53</td>
<td>1.39</td>
<td>0.37</td>
<td>102</td>
<td>Ave</td>
<td>fex</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>5.15</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>0.07</td>
<td>0.52</td>
<td>1.31</td>
<td>0.36</td>
<td>131</td>
<td>Ave</td>
<td>veh</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>7.31</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>0.07</td>
<td>0.42</td>
<td>1.36</td>
<td>0.37</td>
<td>105</td>
<td>Ave</td>
<td>fex</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.01</td>
<td>4.77</td>
<td>SEM</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Fasting Blood Glucose

Serum markers indicated an effect of drug on fasting blood glucose (FBG) ($F=7.849$, $df=1.66$, $p=0.007$). No main effect of diet was observed but a drug:diet interaction was noted ($F=3.914$, $df=2.66$, $p=0.025$) (Table 3). A follow up one-way analysis revealed no significant effect of drug ($p>0.05$), 45% lard and omega-
3 fexofenadine-administered mice experienced a decrease in FBG compared to control (F=7.440, df=1,23, p=0.012 and F=8.546, df=1,21, p=0.008, respectively).

**Analysis of Serum Triglycerides**

A two-way ANOVA revealed no main effect of diet, drug, nor a diet:drug interaction on serum triglycerides (p>0.05).
CHAPTER V
DISCUSSION

The overall goal of this study was to determine the effects of dietary fat and fexofenadine on body weight, metabolic disturbances, and hepatic fat accumulation in mice. Further, it aimed to evaluate the impact of omega-3 fatty acids on the prevention of high fat diet- and/or fexofenadine-induced body weight gain, metabolic disturbances, and hepatic steatosis.

Repeatedly, it has been demonstrated that chronic high fat diets induce pathogenic changes in body weight, liver weight, hepatic fat accumulation, epididymal fat weight as well as elevated serum markers of liver injury (Tarantino, Savastano, & Colao, 2010; Oliveira et al., 2006; Kim et al., 2004). In the present study, no significant differences were observed in average body weight (aBW) between any of the diets and no main effect of time was detected for any of the diets ($p > 0.05$). While this result was somewhat unexpected, previous studies that have yielded significant diet-induced obesity have employed longer durations of high fat diet models such as 12, 48, and 60 weeks compared to the four-week duration of this study (Tarantino, Savastano, & Colao, 2010; Oliveira et al., 2006; Kim et al., 2004).

Fexofenadine, an $H_1$ antihistamine, is a regularly prescribed for the treatment of seasonal allergies. Not only is $H_1$R chiefly involved in allergic reactions and appetite regulation, but $H_1$R knockout mice also exhibit glucose
intolerance and abdominal adiposity, supporting the notion that histamine signaling through H₁R influences carbohydrate and lipid metabolism while also regulating obesity (Wang et al., 2010). In humans, chronic use of H₁ antihistamines is reported to increase body mass indices (BMIs), body weights, waist circumferences, and insulin concentrations (Ratliff, Barber, Palmese, Reutenauer, & Tek, 2010). In the nine-day timeframe of study one, our data indicated that fexofenadine increased body weights in high fat diet-fed mice. In contrast, study two data showed that administration of fexofenadine initially decreased body weights in the high fat and omega-3 diets with weights leveling off by the end of four weeks. One consideration is that weight gain might have ensued if the study had progressed to a longer timeframe.

In addition, 45% lard-fed and omega-3 fed mice demonstrated significantly increased fat masses, consistent with Raveendran et al.’s (2014) conclusion that high fat diet-fed, wild-type mice experienced increased deposition of gonadal fat. The distinction between this study and Raveendran’s (2014) is that an interactive effect of drug and diet was found by Raveendran but no such interaction was discovered in the current study.

In study two, fexofenadine-treated mice demonstrated no significant changes in liver or kidney weights. In retrospect, the study timeframe may have been inadequate to ascertain organ weight fluctuations. Future studies would benefit from longer timeframes to allow time for development of physiological
Regarding blood glucose levels, an initial two-way analysis indicated that fexofenadine reduced FBG values ($F=4.154$, df=1,36, $p=0.049$). A follow up one-way analysis, however, showed that fexofenadine reduced FBG levels in 45% lard and omega-3 fed mice ($F=4.154$, df=1,36, $p=0.049$, and $F=4.154$, df=1,36, $p=0.049$, respectively).

In terms of dietary omega-3 fatty acids, numerous studies have found a link between disproportionate essential fatty acid (EFA) intake ratios and steatosis (Depner et al., 2013; Levy, Clore, & Stevens, 2004; Harris & Bulchandani, 2006). In fact, a reduced omega-6 to omega-3 ratio, achieved through greater intakes of omega-3 fatty acids, has evidenced suppression of various diseases, including inflammatory-based disorders (Simopoulos, 2002). The protective effects of omega-3 fatty acids are thought to stem directly from antioxidant and anti-inflammatory mechanisms. Previous research has shown that all PPAR receptor subtypes bind EPA (Martin, 2010). Therefore, one hypothesis is that omega-3 fatty acids may modulate fatty acid oxidation through interactions with PPAR. Specific mechanistic activities related to the triglyceride-lowering effects of EPA and DHA via PPAR, however, are still awaiting clarification. The literature provides persuasive evidence that omega-3 fatty acids are fundamental dietary supplements for NAFLD patients due to their influence on blood lipid levels and reductions in hepatic fat accumulation (Lalia
& Lanza, 2016). In study one, we found an absence of weight gain in both the vehicle- and fexofenadine-treated omega-3 fatty acid groups, providing support for our secondary hypothesis regarding the role of omega-3 fatty acids in protecting against weight gain. Not only was weight gain significantly less in the omega-3 fatty acid-fed mice than the 45% lard mice, but, in fact, it did not differ significantly from control. Evidence-based recommendations for omega-6 to omega-3 fatty acid ratios for disease suppression in humans fall between 4:1 and 1:1 (Simopoulos, 2002). Achieving this ratio may reduce antihistamine-induced metabolic disruptions. In the future, supplementing antihistamine-treated/high fat diet-fed mice with omega-3 fatty acid levels to reach recommended ratios could provide a model for testing this hypothesis.

In spite of promising omega-3 related results in study one, we encountered unexpected effects in the omega-3-fed group in study two. As previously noted, body weights dropped initially and then subsequently returned to baseline in fexofenadine-treated omega-3-fed mice. This difference from study one may be related to a significant difference in study run-in times. Study one incorporated a one to two week run-in time allowing for mouse acclimation to study conditions. Study two, however, only provided a two-day run-in period. One consideration is that mouse stress may play a role in initial body weight decreases.
In summary, the interactive effects of a high fat diet and chronic fexofenadine administration influence multiple mechanisms that may promote metabolic disruptions such as weight gain, hepatomegaly, and increases in perigonadal fat pad mass. H₁ antihistamines are used by millions of people for controlling histamine-mediated allergic symptoms. Scant research exists, however, examining the interactive effects of routine H₁ antihistamine use combined with a high fat diet. This, along with an escalating prevalence of NAFLD, presents a compelling public health issue which necessitates an improved understanding of the relationship between diet, chronic H₁ antihistamine usage, weight gain, and metabolic disorders.

Although there are currently no medical therapies available for NAFLD, the recommended course of treatment advocates lifestyle changes targeted primarily at weight loss. The role of nutrition in the prevention and treatment of metabolic disorders -- including steatosis -- is paramount. In the future, research aimed at the development of NAFLD treatments will benefit considerably from an emphasis on diet and nutrition. Future studies assessing potential metabolic effects of drugs should consider using a defined run-in period and testing low fat and high fat conditions that cover the range of dietary conditions consumed by humans.
References


The American Heart Association/Metabolic Syndrome. (n.d.). *Heart.org.* Retrieved from http://www.heart.org/HEARTORG/Conditions/More/MetabolicSyndrome/Metabolic-Syndrome_UCM_002080_SubHOmePage.jsp


doi:10.1016/j.cmet.2007.12.009


Spadaro, L., Magliocco, O., Spampinato, D., Piro, S., Oliveri, C., Alagona, C., ...


Williams, C. D., Stengel, J., Asike, M. I., Torres, D. M., Shaw, J., Contreras, M., ...

