Histamine and Obesity: Do Histamine and Histidine Injections Increase Body Weight and Adipose Tissue Mass?

Ganader H. Almansoori

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HISTAMINE AND OBESITY: DO HISTAMINE AND HISTIDINE INJECTIONS INCREASE BODY WEIGHT AND ADIPOSE TISSUE MASS?

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

Ganader H. Almansoori
Indiana University of Pennsylvania
May 2019
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High histamine concentrations in foods may induce a food intolerance and weight gain, but it is not clear if this is from local effects of histamine in the gastrointestinal tract or systemic effects of histamine absorbed and distributed around the body. These studies tested the systemic hypothesis by administering intraperitoneal injections of histamine or its precursor histidine for four weeks to female or male mice fed a low (10%) or high (45%) fat diet. Sub-chronic histamine or histidine resulted in no weight gain in female mice but caused weight loss in male mice fed the 45% fat diet. Histidine-injected mice had significantly reduced perigonadal fat pad mass versus controls, as did histamine-injected male mice. Serum triglycerides were increased in vehicle-injected mice fed the 45% fat diet, but were reduced in histamine or histidine-injected mice fed the 45% fat diet. These results do not support the systemic histamine hypothesis.
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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.

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CHAPTER I

INTRODUCTION

Histamine is a biogenic amine (Figure 1), IUPAC name 2-(1H-Imidazol-4-yl)ethanamine), first discovered in the early 20th century by Sir Henry Dale while he was examining contaminants in ergot extracts. It is naturally synthesized in organisms ranging from bacteria to humans. In the human body, naturally produced endogenous histamine has many functions including acting as an inflammatory mediator (e.g., allergies), a vasoactive substance (a vasodilator) and a neurotransmitter in the brain. Histamine is enriched in some types of foods such as wines, cheeses and fish contributing to dietary intake and in some people to food allergies or intolerance.

Fig. 1. Structure of histamine or 2-(1H-Imidazol-4-yl) ethanamine. Source ChemSpider.

Histamine in the human body may come from external sources, such as histamine-rich foods, as well as the internal sources in which cells synthesize histamine from the amino acid histidine. For many people, histamine-rich foods may be well tolerated, but some people may experience negative reactions to histamine-rich foods including food allergies or food intolerance. Food intolerance refers to a condition in which a person may have a reduced ability to digest or metabolically handle a nutrient, vitamin or food additive. Individuals with food intolerance often experience signs/symptoms related to the gastrointestinal (GI) tract such as nausea, intestinal gas, diarrhea, and constipation. Some
with food intolerance may experience dermal responses such as skin rashes or urticaria, and/or respiratory reactions such as congestion or unproductive cough. Lactose intolerance is an example of a widely known food intolerance. In contrast, a food allergy refers to an exaggerated response immune-mediated reaction to a food that may be moderate to severe or even life threatening in some cases. Symptoms of food allergy may include GI, respiratory or skin manifestations and may result in anaphylaxis.

For histamine, the difference between food intolerance and food allergy can be somewhat confusing because of the presence of high levels of histamine in some foods and the role of endogenous histamine in inflammatory responses and allergies. There are some situations that stimulate and increase suddenly the produce of histamine in human body, which is related to “true” food allergies. In this case histamine at the same time shows an allergic reaction. Many people they think that a true allergic reaction and histamine intolerance are the same, but the truth is, they are two different reactions. The histamine intolerance happens because of eating type of food that have naturally high-level histamine, for example, tomato, nuts, soy, aged cheese, or wine. The people who are most likely to have histamine intolerance are the people who have low level of the metabolic enzymes (diamine oxidase, DAO, and histamine N-methyltransferase, HNMT) that breakdown histamine into less active metabolites (described in literature review). In these cases, because of lower levels or activity of the DAO and HNMT enzymes, they will breakdown the histamine in body more slowly and can then build up high concentrations of histamine and cause symptoms on the human body.\(^8\)
Foods with high concentrations of histamine or high histamine level foods (HHLFs) are of concern because of potential contribution to histamine intolerance\textsuperscript{52} and food allergies and obesity discussed in health blog posts and discussion websites\textsuperscript{50,51}. These websites cite no published evidence, but imply that HHLFs can increase histamine in the GI tract and/or the blood and thereby disrupt normal processes and promote weight gain and obesity\textsuperscript{50, 51, 52}. One even suggested taking the following course of action

“Many people find much success in taking histamine intolerance supplements and natural antihistamines. Since your symptoms are being caused by excess histamine, why not take a supplement that specifically eliminates histamine? It’s really that simple and doesn’t have to be as expensive and risky as OTC drugs.”\textsuperscript{50}

The efficacy and safety of such an action is unknown and there is no legal requirement in the United States for natural supplement manufacturers to prove safety and efficacy.

Concern over HHLFs is part of a larger body of public concern about the possibility of food intolerance or food allergies in general causing weight gain and obesity, popularized with anecdotal evidence on health promotion websites\textsuperscript{53,54} and by television/web personalities such as Dr. Oz\textsuperscript{56} and Dr. Mark Hyman\textsuperscript{55}. In an online article, Dr. Hyman refers to a study that was published in 2007 that examined lipopolysaccharide (LPS), inflammation and obesity in a mouse model but he provided reference or citation which makes it impossible to critically evaluate the evidence\textsuperscript{55}. Searches of PubMed have revealed very few studies that have directly tested hypotheses related to food allergies or food intolerance (including histamine intolerance) and weight gain/obesity. One review examined evidence of imbalance in the intestinal microbiome and its relationship to allergic disease and obesity including some references to diet and food contributions\textsuperscript{57}, but
it did not specifically address food allergies and obesity. Another review\textsuperscript{58} addressed intestinal permeability as a contributor to intestinal inflammation and obesity and provided some linkage to food allergy and obesity/weight gain and even discussed histamine release by mast cells and ECL-like cells, but it did not link evidence of histamine release to weight gain.

In contrast, other research in obese women has shown that supplementation with histidine (Histidine supplementation, HS), the synthetic precursor of histamine, results in reduced body mass-index (BMI), waist circumference, fat mass and markers of inflammation (e.g., TNF-\(\alpha\), IL-6)\textsuperscript{6}. A case study in a patient with histamine intolerance noted weight loss, diarrhea, and abdominal pain and who had been diagnosed with anorexia nervosa\textsuperscript{59}. When the subject was placed on a histamine-poor diet, she gained weight and had improvement in all other symptoms. A study in mice found that histidine injections reduced high-fat-diet-induced weight gain, hyperlipidemia, hepatic steatosis and increased fat tissue mass\textsuperscript{7}. So, histamine or its metabolic precursor have been noted to cause weight loss in humans and experimental animal studies.

The results of these published studies\textsuperscript{6,7,59} conflict with the anecdotal evidence cited in television shows and online blogs. However, there have not been systematic studies to test the hypothesis that increased histamine in the diet or in the bloodstream results in weight gain and obesity. Furthermore, if consumption of HHLFs combined in individuals with histamine intolerance does cause obesity, it is not clear whether this effect would be driven by effects of histamine on cells in the GI tract (e.g., effects on microbiome and intestinal processes and integrity) or driven by effects of histamine in systemic circulation (blood) on organs such as liver and brain.
Eating food enriched in histamine or histidine can increase histamine in the GI tract and may result in a small increase in histamine in the blood, but much of the histamine eaten is broken down by metabolic enzymes such as DAO and HNMT. In contrast, administration of histamine or histidine (metabolic precursor) by injection can increase levels of histamine in the body (blood, extracellular fluid). Because dietary uptake of histidine and histamine into the blood stream can be changed by many local and systemic factors (e.g., local intestinal bacterial metabolism, uptake through intestinal cells, metabolism in the liver), direct introduction of histidine or histamine into the body by injections (e.g., intraperitoneal or intravenous) allows for a more direct comparison of the effects of these compounds on the body.

Fig. 2. The expression of Histamine and Histadine in the brain and the whole body and histamine from diet.
CHAPTER II

LITERATURE REVIEW

Histamine in Biological Systems

Histamine is synthesized by removal of the carboxylic acid group from the essential amino acid histidine (Figure 1). In bacteria and humans this is catalyzed by various forms of the enzyme histidine decarboxylase (HDC)\(^2,3\). Cells that express HDC can produce histamine and then store it in intracellular vesicles until stimulation drives the secretion of histamine (Figure 2). Major histamine-producing cells include members of the immune system such as IgE-receptor-bearing mast cells and basophils, as well as neuroendocrine enterochromaffin-like (ECL) cells in the stomach and tuberomammillary neurons in the hypothalamic region of the brain, central nervous system (Figure 2)\(^3,4\). HDC is also expressed in leukocytes and lymphocytes including neutrophils, monocytes, macrophages, and T lymphocytes\(^11\) although these cells tend to be minor producers of histamine compared to major histamine-producing cells such as mast cells\(^3\).

![Histidine to Histamine Reaction](image)

*Fig. 3. Synthesis of histamine from L-histidine.*
Fig. 4. Major and minor histamine producing cells and stimuli that trigger histamine release. Source: Huang, et al., 2018 Ref 3.

After histamine is secreted as an extracellular signaling molecule, the intracellular signaling is terminated by the breakdown of histamine by metabolic enzymes N-methyltransferase (HNMT) and diamine oxidase (DAO) and eventual removal from the body (Figure 3). These metabolic pathways are found in multiple tissues in humans including brain, skin and the gastrointestinal tract.
Histamine Receptors in the Body

There are multiple subtypes of histamine receptors named H1, H2, H3 and H4 (see Table 1) based on ligand binding properties, gene nucleotide sequences and amino acid sequences\textsuperscript{12,39}. All of these receptors are G-protein coupled receptors (GPCRs)\textsuperscript{39}. Recent studies described the development and characterization of genetically modified mice lacking either HDC\textsuperscript{16} or the histamine H1 receptor\textsuperscript{18} and these tools were used to analyze the functions of histamine. For instance, in the HDC gene knockout experiment, the mice exhibited clinical features of hyperleptinemia, visceral adiposity, and decreased glucose tolerance\textsuperscript{17}. Also, the H1R-/− receptor deficient mice fed a high fat diet with showed evidence of leptin resistance and increased appetite and fat deposition. Also, deficiency of H3R gene in mice, also showed glucose intolerance and an obese phenotype mice with high levels of insulin and leptin in the blood\textsuperscript{18}. These studies show that histamine plays and important part in metabolism and energy regulation.
The histamine receptors (H1 through H4) are expressed by cells in various tissues throughout the body (see Table 1) including H1 and H3 receptors brain regions such as, ventromedial hypothalamic nucleus (VMH), cerebral cortex and the thalamus\(^{19,39}\). These receptors of the histamine are targets neuronal histaminergic projections\(^{20}\).

H1 receptors are found in hypothalamus, cerebral cortex, limbic system, hypothalamus and basal forebrain\(^{21,39}\), while H2 receptors are located postsynaptically and found in a high concentration in parts of the limbic system, such as amygdala and hippocampal formation, and in the basal ganglia\(^ {19,39}\). H1 receptors are also expressed in the liver and the stomach\(^ {39}\). H3 receptors are found on the axon terminals of neurons that contain histamine, and function as autoreceptors that modulate (i.e., provide negative feedback inhibition) of release and histamine synthesis\(^ {23}\). There are also high concentrations of H3 receptors are in the nucleus accumbens, the cerebral cortex, striatum, substantia nigra and olfactory tubercles\(^ {24}\). Unlike the other histamine receptors, H4 has not been observed in the rat and mice brains\(^ {25}\).

Brain histamine has many functions, including the regulation of the neuroendocrine system\(^ {26}\), sleep-awake cycles, feeding and drinking behavior\(^ {27}\), all of which are mediated by H1, H2 and H3 receptors\(^ {28}\). For feeding regulation, H1 and H3 receptors are crucial\(^ {29}\). In rodent H1 receptor antagonists have been noted to increase food intake, while H3 antagonists can reduce food intake. On the other hand, H2 antagonists have no effect.
**Table 1 Overview of human histamine receptor subtypes.**

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<th>$hH_4R$</th>
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<td>5q35.2</td>
<td>20q13.33</td>
<td>18q11.2</td>
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<td>Amino acids</td>
<td>487</td>
<td>359</td>
<td>445</td>
<td>390</td>
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<td>Isoforms</td>
<td>at least 20 (65, 66)</td>
<td>&gt;3</td>
<td></td>
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<td>G protein coupling</td>
<td>$G_\alpha_{q/11}$</td>
<td>$G_\alpha_s$</td>
<td>$G_\alpha_{i/o}$</td>
<td>$G_\alpha_{i/o}$</td>
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<td>Constitutive activity</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<td>Signal transduction</td>
<td>PLC↑, Ca$^{2+}$↑</td>
<td>cAMP↑</td>
<td>cAMP↓, Ca$^{2+}$↑, MAPK↑</td>
<td>cAMP↓, Ca$^{2+}$↑, MAPK↑</td>
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<td>Ubiquitous (mainly lung, CNS, blood vessels)</td>
<td>Ubiquitous (mainly stomach, heart, CNS)</td>
<td>Neurons (CNS and PNS)</td>
<td>Bone marrow, hematopoietic cells</td>
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<td>Physiologic relevance</td>
<td>Bronchoconstriction, vasodilation, food intake, sleep-wake regulation</td>
<td>Gastric acid secretion</td>
<td>Neurotransmitter release (→ sleep-wake regulation, attention/cognition, food intake)</td>
<td>Immune responses (→ chemotaxis, IL-, IFN-modulation)</td>
</tr>
<tr>
<td>Pathophysiologica...</td>
<td>Allergic reactions, emesis, sleep-wake disorders</td>
<td>Gastric ulcers</td>
<td>Cognitive impairment, schizophrenia, sleep-wake disorders, epilepsy, pain, etc.</td>
<td>Inflammatory diseases (allergy, asthma, pruritus, arthritis), pain, etc.</td>
</tr>
</tbody>
</table>

* + and ++, extent of constitutive activity; PL, phospholipase; PNS, peripheral nervous system.
In addition, there is some evidence from a recent study that the circadian rhythm of feeding behaviors and food intake that is regulated by H1 receptors and it’s important to develop obesity ³⁰.

In mice and rats the level of the food consumption decreases when H1 activators (agonists) are injected centrally (i.e., into brain). In contrast, injecting the mice with H3 blockers (antagonists) centrally decreases autoinhibition by the H3 receptor and can thereby increase release (secretion) of histamine and increase the level of histamine in synapses and extracellular fluid and decrease food intake as well in rats ²⁸.

Peripheral (systemic) injection of mice with the irreversible HDC inhibitor α-fluoromethylhistidine (α-FMH) can acutely reduce histamine levels in brain and some other tissues but not mast cells ³². Chronic administration of α-FMH can reduce histamine levels in mast cells as well ³². These histamine-depleting properties of α-FMH have allowed it to be used to pharmacologically assess the role of histamine in various functions including feeding and energy homeostasis. Thus use of α-FMH can complement gene knockout studies to study the role of HDC and histamine in bodily function.

A study in rats of the effects of acute injection of L-histidine either systemically (intraperitoneal, ip) or directly into the brain (intrcerebroventricular, icv) revealed that either injection boosted levels of histamine in the brain and temporarily suppressed feeding. The authors also showed that ip injection of α-FMH blocked the conversion of histidine to histamine and attenuated the effects of L-histidine injections on feeding. The authors concluded that injections of L-histidine acutely suppressed food intake by its conversion into histamine in the hypothalamus ³³. Another study found that microinjection of H1 (but not H2) receptor antagonists into the VMH induced feeding during early light but not early
dark parts of the daily cycle indicating that VMH is one of the sites that involve in regulation of appetite in rats. In mice the central administration of the receptor agonist H1 enhanced c-fos-like immunoreactivity within the paraventricular nucleus (PVN) and decreased the food intake.

In summary, the inhibition of the H1 receptor, via gene deficiency or pharmacological blockade in rodents, can acutely increase food intake, supporting the observation that H1 receptor play an important part in the regulation of food intake.

**Histamine Release from Enterochromaffin-like Cells, Mast Cells and Neurons**

Histamine is a ubiquitous messenger molecule. It is the meditator of many cellular and physiological processes such as increases in capillary permeability due to contraction of terminal venules, the contraction of smooth muscles, neurotransmission in the central nervous system and the release of catecholamine from adrenal medulla. In the nervous system, histamine is associated with multiple processes, such as learning, thermoregulation, circadian rhythm and memory. But it is well known to be involved in the pathophysiology of allergic diseases such as allergic rhinitis, asthma and atopic dermatitis. The axons of the histamine neurons originate from a single source, the tuberomamillary nucleus (TMN) in the posterior hypothalamus. Almost all central nervous system (CNS) regions are innervated by TMN. There is also evidence of heterogeneity of histamine neurons based on innervation patterns.

**Effects of Histamine Receptor Agonists & Antagonists on Body Weight and Metabolism**

The different between this two term Agonist and Antagonist is the agonist the receptor is fully activate because of a substance and it bind with it, and the antagonist is
even though the receptor bind with a substance but it doesn’t activate and in some case its block the activity of other agonists, in some case there is a kind of a receptor mix with the agonist and the antagonist in the way that act either as agonist and antagonist this type called selective receptor modulators\textsuperscript{33}.

The other name For H1 antagonist is H1 blockers or H1-antihistamines, this substance that block the action at the H1 receptor of the histamine, the reason of this block is to do a relieve of allergic reactions\textsuperscript{34}, H1 agonist works the opposite way of the H1 Antagonist.

The H2 Antagonist has another names, which is H2RA \textsuperscript{35} and H2 blockers, its classified as substance the block the action of the histamine parietal cell in the stomach and block action of histamine. H2 antagonists as the H1 antagonists both call antihistamine that relieve the allergic reactions, but so of the H2 antagonists functions opposite agonists relatively as receptor antagonists\textsuperscript{36}.

H3 antagonists is unlike H1 and H2 that block the action of the receptor but it cause sedation when the block happen in the brain, H3 receptor location are in the brain and there are inhibited by autoreceptors which found in the histaminergic nerve terminals, as a mediator to release the histamine\textsuperscript{37}.

**Absorption and Distribution of Histamine from the Intestine**

There is a present study, works on the absorption of histamine, the experiment was using sensitive and highly specific method for estimate radioactive (14C)histamine. Into the lumen of an intestinal loop the (14C)histamine was introduced after that estimate in the blood leaving in the general circulation and most of the time the blood leaning the loop.

In the lumen the (14C)histamine was remaining and in the wall of the intestine was
present and estimated as well. In other study, (14C) histamine absorption was examined
from jejunal loops though their arteries was perfused with dextran-saline solution.
Also, the experiment includes, after a meat meal in cats and dogs which histamine was
determined in intestinal contents as well as many types of diets.

A several amount of (14C)histamine was introduced in anaesthetized dogs into the
ligated stomach or the lumen of a ligated intestinal loop, this study determined the from
the ligated part the radioactive histamine in the venous blood coming.

After introducing 5-5000 jug of (14C) histamine, into a 50, Cg into a loop of
duodenum, or loop of jejunum, ileum or colon. In all eight succeeding 15 min samples was
collected during 2 hr of venous blood, radioactive histamine was detected.

The recovery percentage of (14C)histamine in the blood is between0 04 and 3-7
during this period, After the introducing of 10 mg (14C)histamine radioactive was detected
into the stomach in all sample gastric, at so many times venous blood was collected during
the following 4 hr.

A big amount of (14C) histamine was introduce into an intestinal loop was destroy
either om the wall or the lumen, only the recovery was in the venous blood, After two hour
wall of the loop and contents at the end. Depends on the dose differ amount of
(14C)histamine were introduce into a jejunal loop the recovery was exposed. The amount
with 5000 ,jug to 29-42 % while with 5 jug it amounted to about 1 %. So the recovery was
greater of the introduced (14C)histamine into a perfused jejunal loop. After 2 hr of the meat
meal in the dogs and cat, there was found free histamine in the content stomach and much
smaller amount. From the colon the amount of the content found varied greatly. In the
gastric and 'intestinal phases' of gastric secretion the role of histamine as a hormonal agent\textsuperscript{38}.

**Histamine and Energy Homeostasis**

The regulation of the energy intake and expenditure (i.e., energy homeostasis) in the body is the result of a complicated interplay of many factors (e.g., cells in GI, PNS/CNS, endocrine systems, muscle and adipose tissue; hormones, neurotransmitters, cytokines) that impact appetite and satiety as well as the rate and efficiency of metabolic processes. The basic understanding of role of histamine in energy homeostasis and brain pathways has been established and shows relationships to of histamine to orexigenic (feeding promoting) or anorexigenic (feeding inhibiting) pathways\textsuperscript{47} as diagrammed in Figure 4. There is dense histaminergic innervation of key hypothalamic nuclei involved in energy homeostasis and feeding regulation such as the arcuate and ventromedial nuclei\textsuperscript{47}.

Additionally, treatments that increase extracellular levels of histamine, such as the autoreceptor blocker (antagonist) betahistine, can reduce food intake\textsuperscript{47} and result in weight loss in patients taking obesogenic antipsychotic drugs with antihistaminergic properties\textsuperscript{48}. Activation of histamine H1 receptors (H1R) in the ventromedial nucleus can result in a reduction in food intake\textsuperscript{49}. Furthermore, histamine H1 knockout (KO) mice gradually gain weight during early adulthood and show a reduced responsiveness to the important satiety hormone leptin compared to wild-type control mice\textsuperscript{49}. Therefore, histamine and its receptors are expressed in neural circuits involved in energy homeostasis and disruption of histaminergic signaling can disrupt normal regulatory
mechanisms such as leptin responsiveness. Also, histamine may play as lipolysis accelerator.

![Histaminergic neural pathways and relation to feeding circuits](image)

Fig. 6. Histaminergic neural pathways and relation to feeding circuits\(^7\).

**Evidence of Histamine Effects on Weight: Anecdotal Evidence and Case Studies**

A number of people have admitted that histamine dose effects their weight and helps to gain weight, by only observing and watching the food that they are eating through the day for a long term, to see the result. For example, Dr. Oz in his show was sharing his own experience by reducing his weight 77 lbs in two years, because he cut or he used low histamine diet to have this result, the concept that made him use this diet could be undiagnosed allergy where it found the good bacteria and it cannot process the food, so what happened is the body quickly response with the inflammation, in the bowl and stomach and near some area in the digestive system\(^6\).

Another example, A man his name is Bill, he lost in 30 days 19 lbs by noticing the allergy reaction from histamine food, such as the runny nose and also gain weight so he started to have a low histamine diet and what happen is he lose weight.
One of the techniques that the immune system used when the body is around something can cause harm to the body is the allergic reaction, so what is really happening when that reactions exposed, is releasing histamine and other types of chemicals into the body, after that release of histamine the allergy symptoms started to appear in the person such as skin, eyes, throat, gastrointestinal tract, lung or nose, causing those infamously unpleasant allergy reactions.

Also, histamine release when the person eats a food which an inflammatory. histamine causes water retention which mean it doesn’t allow the fluid in the body to leak out, which is mean an immediate gain on the body weight on the scale, but that gain only consist water because of the inflammatory response, this gain of water can last or reserved for 24 hours, but the reaction response can last for 72 hours which means it cause harm more than just weight gain.

To avoid the harm of histamine, the body control the histamine response by hormones and cortisol such as testosterone and progesterone and using them to build blocks; to balance the negative affect of the hormone the cortisol release more. The side effect of the unbalance hormones can cause disrupt metabolism, water balance, healthy sex drive, thyroid health and immune response.

The increase of producing cortisol in the body can also lead to major problem, which is having a high sugar level in the blood that increase, leads to the growth of the yeast on glucose sensors that will effected the gut flora and can cause bad effect on the body defense system, the more of the yeast growth the more craving to carbs and sugar and that lead to gain weight and diabetes type 2 and for sure weaken the immune response to balance the intestinal bacteria is thrown off 7.
The system that control the body weight and the food intake is very complicated, hunger and satiety are the key factors of eating behavior which is controlled by many elements, such as neuroendocrine systems, circadian rhythm, environmental factors and the behavioral state, all these elements agree to modify by homeostatic characteristics of energy expenditure and appetite. In eating behavior brain histamine plays an important role in the eating behavior which the loss of appetite and its release during the food intake and count as a satiety signal\(^8\).

Additionally, there are some mechanisms that histamine regulates such as insulin function and glucose uptake. To regulate the diurnal rhythm of food consumption it’s very important to activate H1 and H3 receptors based on Preclinical research. Also, these receptors are responsible of expenditure energy intake and recognized as mediators\(^9\).

**Food Intolerance May have Indirect Effects on Body Mass and Metabolism**

Having a high level of histamine in the bloodstream is the definition of the condition of histamine intolerance, the core of this problem to have the symptoms which is lack of the amounts enzyme diamine oxidase, which is produce in the body, the reason of having this lack of producing this enzyme is the poor nutrition that the person have. The symptoms of the histamine intolerance in the blood can be different from individual to other individual, but it all promotes inflammation and will never stop until eliminated, the symptoms such as gastrointestinal changes, acne, flu-like symptoms, appetite changes and weight gain.

In this kind of cases, can cause water retention which may lead to gained weight. The reason of these symptoms is eating food rich with histamine, so the person must avoid the vegetable that is rich with histamine such as the tomato and spinach, not only the
vegetable, also must review the list of the food that is rich with histamine, such as, wine, yeast, yogurt and cheese, not only food must be avoided, many kinds of medicines have a high percentage of histamine\textsuperscript{10}.

**High Histamine Foods**

There are so many foods that is rich with histamine and cause allergy reaction, it should be avoided, at the figure below has lists of high histamine food.

![High Histamine Foods](image)

Fig. 7. List of the high histamine food.
**Food Intolerance**

Non-allergic food hypercreativity or Non-IgE mediated food hypersensitivity, these two team are known as Food intolerance, which is in certain foods that digestive process can have difficulty. People mostly got confuse between food allergy and food intolerance, the different is in food intolerance does not trigger the immune system, while food allergies does. After eating some kind of foods people suffer digestive problems, even though there is no histamine response and the immune system does not react. Food that cause food intolerance E.g. grains that contains gluten, dairy products, any type of food that can cause intestinal gas buildup such as cabbage and beans.

**Histamine Intolerance**

Histamine intolerance happened because of the imbalanced between the selective release of histamine from certain granulocytes, for example (mast cells, basophils and the synthesis), so what happen to the histamine numbers of breakdown to metabolize by enzyme (DAO) and histamine N-methyltransferase (HNMT)\(^{39}\). After that allergic reaction involve as response cause by anaphylactic degranulation, that is sudden release of pre-formed mediators, that include histamine, throughout the body from mast cell and basophils\(^{40}\).
CHAPTER III
MATERIALS AND METHODS

Subject

The subjects for this project included 60 female C57Bl/6J mice (Mus musculus) at the 1st stage of the experiment, and at the second stage the study include 24 female C57Bl/6J mice (Mus musculus), the breeding colony found at Indiana University of Pennsylvania (IUP). Founders of the colony originally came from Jackson Laboratories (Bar Harbor, Maine, USA). The mice weighed approximately 25 grams at the start of the experiment 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

For the period of five days, mice were maintained on LabDiet 5P00/RMH 3000 (T.R. Last, Cabot, PA). After that beginning of the experiment nt. The mice were injected by histamine and other group were injecting histidine for 28 days, with two of different percentage of diets: a high fat diet (45% of dietary calories from lard), and control diet (10% dietary calories from lard) (Research Diets D12450J, D12451, and D03022403, respectively). The sucrose content of these diets was 10% fat, 45% fat.
Reagents

a. Histamine and Histidine

The run-in period of five days was administered, to allow the mice to get use to handle and injections. After that the experiment started with 28 days, 60 female C57Bl/6J mice were randomly assigned into one of two different diet which is 10% fat dietary and 45% fat dietary, the mice was divided into three groups which is (histamine – Histidine – control) each group contain 20 mice and every two mice was left in one cage, and each group divided in two subgroup with different diet which is 45% and 10% and the mice were injected by histamine and histidine daily and the control group only feed on different fat percentage without injection.

Each day at the 28 days the weight of the mice must be measure.
The study ran for 4 weeks. No significant differences in initial body weights existed between treatment groups. The negative control groups. Mice were weighed each morning prior to treatment in order to determine proper injection volumes.

b. Triglyceride colorimetric Assay Kit (Cayman Chemicals)

This step is the final step after the feeding period to the mice, after sacrificing the mice a process the live and extract the liver serum this process followed with instructions that come with kit.

Sample Preparation

A. Serum

Blood collecting from the mice, for 30 min allow the blood to clot in 25°C, then for duration of 15 min centrifuge the blood in speed 2000x at 4°C, after the separation of the blood and the serum, with the pipette, the top yellow serum only pipette up, the serum
should store in ice. If the assay not at the same day freeze it at -80oC for one month.

There are no needs to dilute the serum before the assay.

![Image of a vial held in a hand]

Fig. 8. Preparation of the liver sample.

**B. Tissue Homogenates**

Before mince the liver in to a small pieces, liver should be weight, from the mince tissues Homogenized 350-400 mg in 100 ml of the NP40 dilution, for 10 min centrifuge at 10000x g at 4oC. After that transfer the whole supernatant to new tubes and store in ice, if the assay not at the same day freeze it at -80oC for one month, the tissues sample need to be diluted of at 1:5 or greater, the dilution of the sample is by using the diluted NP40 substitute assay reagent, before assaying.

**Statistical Methods and Data Analysis**

a. In this experiment we used one-, two- or three-way ANOVA depending on the factors being tested (e.g., drug, diet or time). Time was a repeated measure factor (variable). Following ANOVA, if there were significant main effects of drug, then multiple comparisons (Tukey) for body weight, tissue mass, feeding and triglyceride values, were performed to determine significant effects of histamine, histidine compared to vehicle.
b. Creating a standard curve for triglyceride assay by using Linear regression.

**Data Analysis and Statistical Methods**

The repeated measurement of body weight data was analyzed using SYSTAT Statistical Software (SYSTAT Software, Inc., San Jose, CA) by repeated measures analysis of variance (RM-ANOVA). Time was a within group factor, at the same time diet and drug was between groups factors. The 10% fat diet and the vehicle are the controls for this experiment. Three-way ANOVAs to evaluate the between groups factors which is (Drug and Diet) along with within group factor (Time) that have a repeated measures analyses to do evaluation. There was a significant interaction between drug and time, so it followed up with two-way ANOVAs to find up the effect of drug over the time in each diet. two-way ANOVAs was used for the only one time point measurements (e.g., food consumption, serum triglycerides, tissues weight), for the serum triglyceride ANOVA was used after transfer the values to log to be stable variance for ANOVA.
CHAPTER IV

RESULTS

Study 1 – Female Mice Vehicle, Histamine or Histidine and 45% or 10% Fat

a. Effects of Diet and Drug on Body weight in Female Mice

During four weeks of exposure to histidine or histamine while consuming either a high or low-fat diet, the average body weights of female C57Bl6 mice in all drug treatment groups did not appear to differ significantly from each other (Figure 9). With respect to body weight, three-way repeated measures ANOVA (RMANOVA) revealed a significant main effect of diet (F=79.625; df = 1,54; P<0.001) but no significant main effect of drug and no significant interactive effect of diet and drug. There was a significant effect of week of study (F=38.130; df = 3,162; P<0.001) on body weight and a significant interaction of week and diet (F=10.296; df = 3,162; P<0.001). A follow-up analysis using single degree of freedom polynomial contrasts revealed a significant linear trend of week and diet that was manifested as a steady increase in body weight of the mice fed the 45% fat diet (Figure 9). By the end of week four, the average body weight of female mice fed the 45% fat diet had increased approximately 6 to 10% (Figure 9B)
Fig. 9. Effects of dietary fat and drug (histidine or histamine) on body weight over four weeks of administration to female C57Bl6 mice. Panel A, 10% fat diet. Panel B, 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also, * indicates significant differences from control values.

Since there was not a significant main effect of drug on body weight, the data from diet groups were collapsed such that data from all mice on 10% diets were grouped together (regardless of drug treatment) and data from all mice on 45% diets were grouped together to perform an analysis on the effect of diet alone. The results of this analysis showed a highly significant effect of the 45% fat diet on body weight (gain in body weight) such that by the end of the study, mice on the 45% diet had increased body weight by about 8% on average while mice on the 10% diet were not different than baseline (about 100%) as shown in Figure 10. Statistical analysis showed a significant main effect of diet on body weight ($F=79.625; \text{df} = 1,54; P<0.001$) and a main effect of week ($F=79.625; \text{df} = 1,54; P<0.001$) and an interactive effect of diet and week ($F=79.625; \text{df} = 1,54; P<0.001$).
Fig. 10. Effect of dietary fat (10% or 45%) on body weight over four weeks of administration to female C57Bl6 mice. Note sample sizes are N=30 mice per treatment group. Also, * indicates significant differences in average body weight for 45% high fat diet from 10% low fat control diet.

b. Effect of Diet and Drug on Food Consumption in Female Mice

Food consumption (measured as average grams per day per mouse for each cage of two mice) generally declined from the first to second week and then stabilized in a range between approximately 2.4 and 2.8 grams per day per mouse (Figure 11). Visual inspection of the data suggested there were no apparent differences in food consumption between vehicle, histamine, and histidine treated groups. Statistical analysis (three-way RMANOVA for drug, diet and week) confirmed this showing no significant main effect of drug (P>0.05) but a significant main effect of diet (F=7.037; df = 1,24; P=0.014) and week (F=59.561; df = 3,72; P<0.001). Since there was no effect of drug, the data were collapsed into 10% or 45% fat groups (N=15 for each) and a two-way RMANOVA for the effect of diet and week on average food consumption was performed. The analysis showed a significant main effect of diet (F=7.963; df = 1,28; P=0.009) and week (F=41.089; df = 3,72; P<0.001) and a significant interactive effect of diet by week (F=14.901; df = 3,84;
P<0.001). The interactive effect of diet and week is seen as a significant reduction in average food consumption by the mice fed the 45% diet compared to the mice fed the 10% diet in weeks one and two (Figure 12). Female mice fed the 45% fat diet ate 0.466 g less per day in week one and 0.261 g less per day in week two than did the 10% fat fed mice in the same periods (weeks one and two). During weeks three and four there were no significant differences in food consumption between the 10% and 45% fate fed female mice.

![Graph](image)

**Fig. 11.** Effects of dietary fat and drug (histidine or histamine) on food consumption over four weeks of administration to female C57Bl6 mice. Panel A, 10% fat diet, Panel B, 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also, * indicates significant differences from control values.
Effect of dietary fat (10% or 45%) on food consumption over four weeks of administration to female C57Bl6 mice. Note sample sizes are N=15 cages per diet treatment group. Also, * indicates significant differences in average body weight for 45% high fat diet from 10% low fat control diet.

c. Effect of Drug and Diet on Fat Pad Mass (perirenal, retroperitoneal and perigonadal)

At the end of the study, tissues were collected including three visceral fat pads (perirenal, retroperitoneal and perigonadal), liver and kidneys and weighed (wet weight). There are no significant effects of drug (histamine or histidine) or of diet (10% or 45%) and no significant interaction of drug and diet on perirenal fat pad mass.

There were no apparent differences in the average mass of the dissected perirenal fat pads in female mice (Figure 13) and statistical analysis (two-way ANOVA) confirmed no significant main effect of drug or diet in average perirenal fat pad mass in female mice.
Figure 13. Effects of dietary fat and drug (histidine or histamine) on perirenal fat pad mass in female C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also, * indicates significant differences from control values.

For retroperitoneal fat pads, there was no apparent effect of drug (histamine or histidine) on fat pad mass, but for diet, there was a consistent increase in fat pad mass in the 45% fat fed mice compared to the 10% fat fed mice (Figure 14). Statistical analysis revealed a significant effect of diet (F=7.193; df = 1,54; P<0.01) but not drug. Collapsing the 10% and 45% fate fed mice together, the average retroperitoneal fat pad mass in the 45% (0.126±0.004 g) was significantly greater than the average fat pad mass in the 10% fat fed mice (0.110±0.004 g) as is shown in Figure 15.
Fig. 14. Effects of dietary fat and drug (histidine or histamine) on retroperitoneal fat pad mass in female C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also, * indicates significant differences from control values.

Finally, for perigonadal fat pads, visual inspection showed an apparent general increase in the fat pad mass in the 45% fat fed mice compared to the 10% fat fed mice and within the 45% fat fed mice, there was a decrease in the mass the histidine injected

Fig. 15. Effects of dietary fat on retroperitoneal fat pad mass in female C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=30 mice per treatment group (e.g., vehicle 10% fat). Also, * indicates significant differences from control values.
mice and possibly the histamine injected mice. Statistical analysis revealed a significant effect of diet (F = 20.704; df=1, 54; P < 0.001) and drug (F = 5.285; df=2, 54; P = 0.008) but no significant interaction of drug and diet (P>0.05). One-way ANOVA for drug showed a significant effect of drug on perigonadal fat pad mass (F = 3.746; df=2, 57; P = 0.030) and a post-hoc Tukey test revealed that the perigonadal fat pads of mice injected with histidine were significantly less than the perigonadal fat pads of mice injected with vehicle. Further analysis of drug effects within the 45% fat fed mice confirmed a main effect of drug (F = 9.420; df=2, 27; P = 0.001) and post-hoc Tukey comparisons revealed a significant reduction (about 25%) in perigonadal fat pad mass in mice injected with histidine compared to mice injected with vehicle. Similar analyses within the 10% fat fed mice revealed no significant effects of drugs (histamine or histidine).

Fig. 16. Effects of dietary fat and drug (histidine or histamine) on perigonadal fat pad mass in female C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly different between 10% and 45% and * indicates significant differences from control values.
d. Effect of Drug and Diet on Liver and Kidney Mass

Average wet weights of liver and kidney did not differ between drug treatment groups or diet groups (Figures 17 and 18). Statistical analysis confirmed no significant effects of diet or drug on liver or kidney mass.

Fig. 17. Effects of dietary fat and drug (histidine or histamine) on liver and kidney mass in female C57Bl6 mice fed a 10% or 45% fat diet. Left panel liver, right panel kidney. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.

e. Effect of Drug and Diet on Triglycerides

At the end of the study, serum samples were collected and analyzed for triglycerides.

There is significant effect of diet and drug on serum triglycerides, increase of the body weight on the mice were feeding on 45% comparing to the other group that fed on 10%

Significant effect of diet (F = 11.965; df=1, 54; P = 0.001) and drug (F = 5.557; df=2, 54; P = 0.006) on serum triglycerides (sTG), but there was no significant interactive effect drug and diet on sTG (P>0.05). One way ANOVA for drug in mice fed the 10% diet, showed no significant effect of drug on sTG. However, one way ANOVA for the effect
of drug in the mice fed the 45% diet showed a significant main effect of drug on sTG (F = 7.325; df=2, 27; P = 0.003) and a post-hoc Tukey test revealed that the sTG levels of mice injected with histidine or histamine were significantly less (P <0.05) than sTG levels of mice injected with vehicle. Thus treatment with either histamine or histidine resulted in lower levels of serum triglycerides in mice fed a 45% fat diet compared to vehicle treated mice fed the 45% fat diet.

Figure 18. Effects of dietary fat and drug (histidine or histamine) on serum triglycerides in female C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.

**Study 2 – Male Mice Vehicle, Histamine or Histidine and 45% or 10% Fat**

**a. The Effect of Histamine and Histidine on Food Consumption in Male Mice**

During four weeks of exposure to histidine or histamine while consuming either a high or low-fat diet, the average body weights of male C57Bl6 mice in all drug treatment groups did not appear to differ significantly from each other on 10% fat diet, but at the 45% fat diet there is a significant increase, by comparing the the two group there is a significant increase. Three-way RM-ANOVA showed significant effects of diet (F =
15.553; df=1, 54; P < 0.001), drug (F = 4.439; df=2, 54; P = 0.016) and a significant effect of week (F = 9.134; df = 4, 162; P =0.001) on body weight but no significant interactive of drug and diet (P>0.05). One way ANOVA for the effect of dietary fat in mice treated with vehicle showed a significant main effect of fat (F=11.488; df=1,18; P=0.003). Contrast analysis of effects of fat by week revealed that body weight was significantly higher for mice fed the 45% fat diet than mice fed the 10% fat diet on weeks 1, 2, 3, and 4. Two-way RM-ANOVA for mice fed 10% fat diet revealed no effects of drug or week. For mice fed the 45% fat diet, Two-way RM-ANOVA revealed main effects of drug and week. Contrast analysis showed that mice injected with histidine or histamine had significantly lower body weight than vehicle injected mice on weeks 2, 3 and 4.
Fig. 19. Effects of dietary fat and drug (histidine or histamine) on body weight over four weeks of administration to male C57Bl6 mice. Upper left panel 10% fat diet; Upper right panel 45% fat diet; Lower panel Collapsed 10% and 45% analysis. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat) upper panels; N=30 lower panel. Also, * indicates significant differences from control values.

b. The Effect of Histamine and Histidine on Food Consumption in Male Mice

The food consumption of male mice injected with vehicle or histamine or histidine was indistinguishable (Figure 20) within the mice fed 10% fat or 45% fat diets, although food consumption for the 10% mice appeared to decline over the four weeks of the study. In the 10% fat diet there was a significant decrease on body weight on the male mice, but no change happened through the four weeks in the 45% fat diet on the male mice.
Statistical analysis showed that there is a no significant effect of (Histamine or Histidine) on Food consumption in male mice fed on 10% or 45% fat diet.

Figure 20. Effects of dietary fat and drug (histidine or histamine) on food consumption (g per mouse per day) over four weeks of administration to male C57Bl6 mice. Left panel 10% fat diet; Right panel 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat) upper panels. Also, * indicates significant differences from control values. There was no significant effect of histamine or histidine, but there was an effect of week.

Since there was no effect of histamine or histidine, the food consumption data for dietary fat were collapsed into 10% and 45% fat groups and analyzed. Two-way RMANOVA revealed a significant effect of dietary fat (F=10.863; df=1,28; P<0.001) and week (F=52.155; df=3,84; P<0.001) on food consumption and significant interactive effect of diet and week (F=18.136; df=3,84; P<0.001) on food consumption in male mice. Single degree of freedom contrast analyses indicated that male mice fed the 45% fat diet consumed less food than male mice fed 10% fat diet across all weeks (Figure 21). At the 10% fat diet a dramatic decrease in the body weight in the male mice, but the 45% no significant changes in the body weight. There is a significant effect of fat diet (10% or 45%) and a significant interaction of diet and week on food consumption in male mice.
Fig. 21. Effect of dietary fat (10% or 45%) on food consumption over four weeks of administration to male C57Bl6 mice. Note sample sizes are N=15 cages per diet treatment group. Also, * indicates significant differences in average body weight for 45% high fat diet from 10% low fat control diet. There was a significant effect of diet and week and significant interactive effects of diet and week on food consumption in mice fed 10% or 45% fat diet.

c. **Tissue Analysis: Perigonadal Fat Pad Mass**

At the end of the study, male mice fed the 45% fat diet had perigonadal fat pads that were much larger than male mice fed the 10% fat diet (Figure 22, vehicle groups). In the 45% fat fed mice, those injected with histamine or histidine had less massive perigonadal fat pads (Figure 22, right panel). However, no significant changes happened in the other group the 10% fat diet. For male mice, there were significant effects of histamine and histidine ($F=4.882; \text{df}=2,54; \text{P}=0.011$) and of dietary fat ($F=8.203; \text{df}=1,54; \text{P}=0.006$) on perigonadal fat pad mass. However, there was no significant interactive effect of drug and diet on perigonadal fat pad mass. Mice fed the 45% fat diet had a 21% increase in perigonadal fat pad mass compared to mice fed the 10% fat diet (Figure 22). Male mice fed the 45% fat diet and injected with either histamine or histidine had an approximate 20% reduction in perigonadal fat pad mass compared to mice injected with vehicle.
Fig. 22. Effects of dietary fat and drug (histidine or histamine) on perigonadal fat pad mass in male C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.

d. Retroperitoneal Fat Pad Mass

The retroperitoneal fat pad mass was larger in male mice fed the 45% fat diet compared to male mice fed the 10% diet (Figure 23). There was a modest reduction in the retroperitoneal fat pad mass in male mice injected with histamine or histidine and fed the 45% fat diet compared to vehicle injected mice fed the 45% fat diet. Statistical analysis showed a significant main effect of diet (F=4.882; df=2,54; P=0.011) but no significant main effect of drug (histamine or histidine) and no significant interaction of drug and diet on retroperitoneal fat pad mass. Mice fed the 45% fat diet had a 20.3% increase in retroperitoneal fat pad mass (Figure 23).
Fig. 23. Effects of dietary fat and drug (histidine or histamine) on retroperitoneal fat pad mass in male C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly different between 10% and 45% and * indicates significant differences from control values.

e. Perirenal Fat Pad Mass

For perirenal fat pad in males, there were no significant effects of drug (histamine or histidine) or of diet 45% and on perirenal fat pad mass (Figure 24).
f. Liver Mass

There was no significant effect of drug or diet on liver mass (Figure 25).

![Liver Mass Diagram](image)

Fig. 25. Effects of dietary fat and drug (histidine or histamine) on liver mass in male C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.

g. Kidney Mass

There was no significant effect of diet or drug on kidney mass in male mice (Figure 26).

![Kidney Mass Diagram](image)

Fig. 26. Effects of dietary fat and drug (histidine or histamine) on liver mass in male C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.
h. Analysis of Serum Triglycerides

There was a significant increase in the serum triglycerides on the 45% diet in male mice injected with vehicle (Figure 27). For male mice fed the 45% fat diet, serum triglyceride levels were much lower in the mice injected with either histamine or histidine compared to the mice injected with vehicle (Figure 27). Statistical analyses revealed a significant effect of diet (F = 11.965; df=1, 54; P = 0.001) and drug (F = 5.557; df=2, 54; P = 0.006) on serum triglycerides (sTG), but there was no significant interactive effect drug and diet on sTG (P>0.05). One-way ANOVA for drug in mice fed the 10% diet, showed no significant effect of drug on sTG. However, one-way ANOVA for the effect of drug in the mice fed the 45% diet showed a significant main effect of drug on sTG (F = 7.325; df=2, 27; P = 0.003) and a post-hoc Tukey test revealed that the sTG levels of mice injected with histidine or histamine were significantly less (P <0.05) than sTG levels of mice injected with vehicle.

![Fig. 27. Effects of dietary fat and drug (histidine or histamine) on serum triglycerides in male C57Bl6 mice fed a 10% or 45% fat diet. Note sample sizes are N=10 mice per treatment group (e.g., vehicle 10% fat). Also note, # indicates significantly difference between 10% and 45% and * indicates significant differences from control values.](image)
CHAPTER V

DISCUSSION AND CONCLUSION

The overall goal of this study was to determine effect of histamine and histidine injection on increasing the body weight of the male and female mice. Repeatedly, it has been demonstrated that in mice the histamine or histidine injections reduced high-fat-diet-induced weight gain, increased fat tissue mass, but at the recent study that there is no weight gain with histamine or histidine, weight loss in males on 45% fat diet and histamine or histidine, and there is no weight loss in females up to 4 weeks, reductions in perigonadal fat pad mass with histidine in males and females, also reductions in perigonadal fat pad mass with histamine in males only.

It shows also in the serum triglycerides increased with 45% fat diet (vs 10% fat), The mice that were fed on high fat diet and injected with histamine or histidine had reductions in serum triglycerides (males and females). In other study, for 20 h Kun Ming mice were exposed to restraint stress, in cerebral regions and plasma both there was significant increase. In addition, when the mice were injected with lipid emulsion, the period of elimination was prolonged in the restraint group in the plasma triglyceride. At 35 min after the lipid emulsion administration in the stress group the Plasma triglyceride was 523 ± 44 mg/dl, at the other hand in the restrained mice it was 436 ± 41 mg/dl which these mice were giving histamine dose of 50 mg/kg. The mRNA expression in response to histamine and the significant up-regulated hepatic triglyceride lipase (HTGL) activity show and explain the improved plasma triglyceride metabolism. As a result, in mice the effect of stress-induced histamine on lipid metabolic disorder loaded with promotion of lipase
activity and restraint stress arose from its anti-stress action\textsuperscript{41}. So, the study explain the reason behind the decrease in body weight of the mice and triglyceride.

It is not clear why HS treatments, which can increase histamine levels in the body should have opposite effects to HHLFs. It is possible that histamine produced from the histidine precursor by cells leads to very different effects on energy balance than does histamine increased in the blood from diet or injections. Because dietary uptake of histidine and histamine into the blood stream can be changed by many local and systemic factors (e.g., local intestinal bacterial metabolism, uptake through intestinal cells, metabolism in the liver), direct introduction of histidine or histamine into the body by injections (e.g., intraperitoneal or intravenous) allows for a more direct comparison of the effects of these compounds on the body.

In this present study, this does not support the hypothesis that the project is trying to prove, and it’s also against the follow article that Dr. Scott Rollins suggest about the relationship between food intolerance and weight gain, so the article suggested three reason that can lead to gain weight because of the food intolerance, which is, First, the people are craving the food that they are allergic to. High-glycemic food and sugar, they have in common allergens for example corn and wheat, this kind of food will continually make the patient feel good cause this food stimulate this process call neurotransmitter and it happens in the brain called serotonin. Also, beside this allergy effect the serotonin decrease because of inflammatory cytokines which lead to complicate the issue of cravings.

Secondly, the cortisol which is the anti-inflammatory hormone, which is the connection between weight gain and food allergies. Naturally the body to control inflammation it makes cortisol, but at the other hand the cortisol it makes the blood sugar
goes up and cause a food allergy chronically, the inflammation will increase the cortisol and as a result of this increasing the blood sugar will increase as well. So that lead to have more insulin resistance and diabetes, and cortisol will cause the buildup of the fat around the midsection which lead to an apple shaped body and as a result leads to weight gain.

Third, lack of nutrition in the food allergies can cause weight gain which lead to the result of an overworked immune system and an inflamed gut. The gut wall inflammation, usually combined with the positive bacteria that can lead to lack in nutrients that are for a proper weight control and metabolism. Also, let’s keep on mind that to operate effectively the immune system needs nutrients, so what happen to fight the immune reaction the chronic activity uses a lot of nutrients just to keep up trying to fight42.

As a conclusion the histamine in the blood is not the histamine that causes effect on the body weight or the tissue but the histamine that the mice through the diet and reach to the gut is the histamine that effect the body weight and tissue that could be because of the micro-organism in the gut, and also as a conclusion this does not support histamine-driven weight gain hypothesis, and did not test histamine tolerance hypothesis.
Fig. 28. Breakdown of histamine in the gut by the metabolic enzymes DAO and HNMT in healthy individuals, and people with histamine intoxication or histamine intolerance. Source Ref 61.
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