# Comparison of Levels of the Inflammatory Cytokines Tumor-Necrosis Factor-α and Interleukin-1 in the Pancreases of Rats with Homozygous and Heterozygous Inactivation of the Melanocortin Receptor Gene Compared to Wild-Type Rats

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**Abstract**

One of the symptoms of obesity is chronic inflammation of the tissues.[1] This inflammation is caused primarily by macrophages in adipose (fat) releasing pro-inflammatory cytokines.[1] Alpha-melanocyte stimulating hormone binds to melanocortin receptors on the surface of macrophages to decrease this inflammatory response.[1] Rats without a functional melanocortin receptor are obese.[2] My hypothesis is that rats with the melanocortin receptors completely inactivated (homozygous) will have much higher levels of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) than rats

with the melanocortin receptors intact (wild-type), and that rats with the melanocortin receptors partially inactivated (heterozygous) will have slightly higher levels of the inflammatory cytokines than wild-type rats. To test the hypothesis, I compared levels of two inflammatory cytokines in pancreatic tissue samples from groups of eight homozygous, heterozygous, and wild-type rats. The protein levels of the samples were normalized using a Bradford Assay, and then an ELISA was run to determine the concentrations of TNF-α and IL-1. There were no statistically significant differences among the rat groups for either cytokine. Therefore the working hypothesis was rejected.

## Introduction

How does the amount of inflammatory cytokines in the pancreas of an obese rat compare to the amount in the pancreas of a lean rat? This question is important because increasing numbers of people are being diagnosed with obesity and type II diabetes. The results of this project could make a difference to people who have type II diabetes due to obesity because doctors could better understand how the two are related and how to treat them.

### a. Inflammation

TNF-α and IL-1 are produced by many leukocytes, including activated macrophages.[3] TNF-α strongly mediates inflammatory and immune functions.[3] It also regulates the growth of many cells, promotes angiogenesis, suppresses lipogenic metabolism, and induces macrophages and endothelial cells to

produce chemokines, which draws more inflammatory cells into the area.[3,4] It is detrimental at high concentrations and may lead to high fever or shock.[3]

Inflammation is a response of the body to many stimuli, such as infection or injury.[4] Acute inflammation is rapid and is accompanied by a systemic reaction called the acute-phase response.[4] The acute-phase response is characterized by a quick change in levels of many proteins in the plasma.[4] Chronic inflammation can lead to pathologic consequences.[4] (See Appendix A for more background information.)

**b. Inflammatory Cytokines and Obesity**

Hotamisligil and colleagues reported that adipocytes, or fat-filled cells, express TNF-α, which is a proinflammatory cytokine.[3] In obese animals, this expression of TNF-α is significantly increased.[3] TNF-α interrupts the intercellular signaling pathway after insulin binds to its receptor on the cell surface, thus inhibiting insulin sensitivity.[3] Another inflammatory cytokine, IL-6, also interrupts the intercellular pathway in hepatocytes.[3] It has been noted that obesity can cause inflammation, leading to type II diabetes (NIDDM).[3]

Normally, tyrosine kinase on the interior part of the insulin receptor (Receptor Tyrosine Kinase or RTK) is activated when insulin binds.[3] It will autophosphorylate and phosphorylate some nearby proteins.[3] Adaptor proteins must bind to phosphorylated tyrosine sites on the receptor to start the signaling cascade.[3] TNF-α stops the autophosphorylation and promotes phosphorylation of the serine residues of the receptor and the Insulin Receptor Substrate 1 (IRS-

1) adapter in adipocytes.[3] This keeps the tyrosine residues from becoming phosphorylated.[3]

Inflammatory cytokines bind to the surface receptors to signal activation of kinases.[3] These kinases phosphorylate IRS-1 on serine residues, thereby inhibiting it.[3] Inflammatory cytokines draw macrophages as well as other immune cells into adipose tissue.[3] It has been suggested that Toll-like receptors (TLR) on macrophages recognize excess nutrients and initiate the inflammatory response.[3] To support this, TLR4 and TLR2 respond to high levels of free fatty acids.[3] Additionally, there is an increase in lipopolysaccharide, an inflammatory molecule, in the blood in mice after feeding.[3] This may be due to the increased permeability in the intestine, which allows inflammatory molecules to go into the circulation.[3] It is possible that animals living in a nutrient-rich environment characteristic of obesity have a long-lived inflammatory response due to the increased permeability of the intestine because the permeability is elevated for a long period of time after a meal is completed.[3]

Hypoxia, low levels of blood oxygen, resulting from expansion due to obesity may also trigger adipose tissue to release inflammatory cytokines.[3] Hypoxia due to expansion is caused by rapid growth with which the vascular system cannot keep pace.[3] This causes an ischemic state in which organs receive less blood oxygen.[3] This may cause strokes or heart disease.[3] Macrophages in ischemic tissues express proinflammatory proteins while angiogenic proteins signal an increase in production of blood vessels.[3]

Obese animals have a positive feedback loop.[3] Full adipocytes leak fatty acids into the blood, which induce more inflammation.[3] These cells also go through endoplasmic reticulum (ER) stress where the ER accumulates large amounts of unfolded proteins.[3] This also promotes the release of inflammatory cytokines.[3]

**c. The Pancreas, Hormones, and Digestive Enzymes**

The pancreas is an endocrine and an exocrine gland.[5] Inflammation and damage to the pancreas may inhibit both types of secretions, affecting both the digestive process and blood sugar regulation. Most of the exocrine cells produce digestive enzymes and are arranged in clusters called acini.[5] The islets of Langerhans are found within the acini and produce peptide hormones.[6] There are three types of cells in the islets: α cells produce glucagon; β cells, insulin; and δ cells, somatostatin.[6]

The pancreas is connected to the small intestines by two ducts, the pancreatic duct and the accessory duct.[5] Pancreatic juice contains several digestive enzymes and buffers the gastric juices to the proper pH for the small intestine.[5] The digestive enzymes include pancreatic amylase, which breaks down starches; trypsin, chymotrypsin, carboxypeptidase, and elastase, all of which break down proteins; pancreatic lipase, which breaks down triglycerides; and ribonuclease and deoxyribonuclease, which break down RNA and DNA.[5]

Insulin is secreted when the blood glucose level is high. It signals cells to take up sugar.[6] Glucagon signals the liver to produce more blood glucose when

blood sugar levels are low.[6] The secretion of glucagon is stimulated by exercise and a rise in circulating amino acids, which occurs with low blood glucose levels.[5] Glucagon also stimulates the release of insulin.[5] Insulin, by contrast, inhibits the release of glucagon.[5] Human growth hormone (hGH) and adrenocorticotropic hormone stimulate the release of insulin.[5] Acetylcholine, arginine and leucine, and glucose-dependent insulinotropic peptide (GIP) stimulate the release of insulin, as well.[5] Acetylcholine is a neurotransmitter that innervates the pancreatic islets.[5] Arginine and leucine are amino acids that would be present after a high protein meal.[5] GIP is a hormone released by cells in the small intestine.[5]

Hyperinsulinism usually occurs when too much insulin is injected into a diabetic.[5] This causes hypoglycemia, low blood sugar.[5] The low blood sugar triggers the release of epinephrine, glucagon, and hGH. If the hypoglycemia is too severe, mental disorientation, convulsions, unconsciousness, and shock will occur.[5] This shock is termed insulin shock.[5] Death can occur if blood glucose is not brought back to normal levels.[5]

Pancreatic somatostatin inhibits secretion of glucagon and insulin, slows the absorption of nutrients from the gastrointestinal (GI) tract, and inhibits the release of growth hormone.[5] Pancreatic polypeptide inhibits the secretion of somatostatin, gall bladder contraction, and the secretion of digestive enzymes from the pancreas.[5]

Alpha-melanocyte stimulating hormone binds to melanocortin receptors on the surface of the macrophage to decrease this inflammatory response.[1] The leptin-melanocortin system has a primary function of conserving energy.[2] Rats with without a functional melanocortin receptor eat more food, have lower activity levels and have higher levels of leptin and insulin than rats with functioning melanocortin receptors, and thus are predisposed to being obese.[2]

**The Objectives and Methodology of this Project**

The objective of this project is to compare the concentration of TNF-α and IL-1 in the pancreas of knock-out rats, which are rats with either fully or partially inactivated melanocortin receptor genes, with those of wild-type rats. This will allow for a

comparison of the amount of inflammation of the pancreas of rats genetically predisposed to obesity (knock-out rats) to that of rats genetically predisposed to leanness (wild-type rats).

The working hypothesis is that the amount of TNF-α and IL-1 will be increased in the pancreas of the rats in which the gene is fully knocked-out and only slightly increased in the pancreas of the rats in which the gene is partially knocked-out compared to that of the wild-type. This is based on research that shows that adipose cells tend to express more TNF-α and IL-1.[5] The null hypothesis is that there is no change in the amount of TNF-α or IL-1 in the pancreas of the knock-out rats compared to that of the wild-type rats.

To test the hypothesis, a Bradford Assay was run to normalize the concentration of protein in the portion of pancreas tested. The Bradford reagent contains Coomassie Blue dye, which binds to amino acids with basic or aromatic side chains. When the dye binds to these amino acids, it changes color. This color change can be measured in a spectrophotometer, and the concentration of protein can be determined by comparing each sample to a standard curve.

Enzyme-linked immunosorbent assays (ELISAs) were performed to determine the concentrations of TNF-α and IL-1. They can be used to find whether there is a specific molecule in a sample and its level.[6] First, the sample and serial dilutions of the standard control are put on an inert surface where the proteins are absorbed.[6] Then the surface is washed with a block protein.[6] The block protein is inexpensive, nonspecific, and is used to prevent proteins from other steps from being absorbed onto the surface.[6] Next, the surface is treated with a primary antibody that binds the specific protein of interest.[6] After the unbound primary antibody is washed away, a secondary antibody to the molecule is introduced.[6] This secondary antibody will bind to the primary antibody and is linked to an enzyme that will produce a color change by reacting with its substrate.[6] The unbound secondary antibody is washed away before the substrate is added.[6] The concentration of the protein of interest in the sample is proportional to the color intensity as determined by a spectrophotometer.[6] A specialized plate reader measures the color intensity and compares it to a standard curve generated with the same reactions.[3]

A sandwich ELISA detects and measures antigens.[3] The capture antibody is on the well surface.[3] The sample is added, and the protein of interest will bind to the antibody.[3] The sample is washed away before the secondary antibody is added.[3] This secondary antibody will bind to a different part of the protein and is linked to an enzyme.[3] After this antibody is washed away, the substrate is added.[3] Alternatively, an enzyme-linked secondary antibody, a biotin-linked antibody may be used.[3] Biotin binds to avidin. After the excess is washed away, enzyme-linked avidin is added, followed by the substrate, which will undergo a color change in the presence of the enzyme. Finally, a stop solution is added to end the reaction. The resulting color change is quantified using a plate-reading spectrophotometer and analyzed by linear regression analysis.[3] Sandwich ELISAs have been useful for measuring the concentration of a soluble cytokine in tissues.[3] This is the method that we utilized (See Appendix B). This type of test was used because it can directly measure the concentration of a target protein.

## Methods and Materials

### d. Homogenizing the pancreas

A small portion of pancreas, roughly 5 mg, was portioned into a Dounce Homogenizer. Three hundred microliters of lysate buffer solution was added to the homogenizer, and the portion of pancreas was homogenized with 3-4 twists. The homogenized pancreas solution was placed in an Eppendorf tube and agitated in an ice bath for 3 hours. The sample was then centrifuged for 20 minutes at 12,000 rpm at 4˚C. The

supernatant was decanted and the pellet was discarded. The supernatant to be tested was stored in the refrigerator until analyzed.

**e. Quantifying protein levels**

A Bradford Assay was run on each sample using a series dilution of 1:3, 1:10, 1:30, and 1:100. Further assays were run if the concentration was too high. Five microliters of sample or standards (TBS blank, 1 mg/mL of BSA, 0.5 mg/mL of BSA, 0.25 mg/mL of BSA, and 0.125 mg/mL of BSA) were placed into the wells of a 96-well plate. Two-hundred fifty microliters of Bradford reagent were then added to each well. The plate was put on a shaker to be gently shaken for approximately thirty seconds. The plate was allowed to stand for about 5 minutes, and bubbles in the wells were popped. The plate was read at 595 nm. The Bradford Assay was used to determine the concentration of proteins in each sample.

**f. Normalizing the samples**

The results of the Bradford Assays were used to calculate how much to dilute each sample so that the protein concentrations of the samples were the same. The samples were diluted with 1x sample buffer diluent.

**g. IL-1 ELISA**

An AbCAm kit was used in accordance with the manufactor's instructions. Eight three-fold dilutions of the IL-1 standards were prepared. The samples of pancreatic lysates and the standards were placed in the plate in duplicate. The test plate was covered and incubated overnight on a

shaker in the refrigerator. The solution was discarded, and the plate was washed four times with wash solution. One hundred microliters of biotinylated IL-1 detection antibody was added. The plate was covered and incubated for 1 hour at room temperature. The solution was discarded and the plate was washed four times with wash solution. One hundred microliters of HRP-streptavidin solution was added to each well. The plate was covered and incubated for 6.5 hours at room temperature on a shaker. The solution was discarded, and the plate was washed four times with wash solution. One hundred microliters of TMB One-Step Substrate Reagent was added to each well. The plate was incubated for 30 minutes at room temperature in the dark on a shaker. Fifty microliters of stop solution was then added to each well. The plate was read at 450 nm on a plate-reader.

**h. TNF-α ELISA**

An AbCAm kit was used and performed as described above for IL-1 determination. The sample size varied for the TNF-α ELISA due to lack of sample or sufficient numbers of wells for the ELISA plate.

## Results

### a. IL-1 ELISA

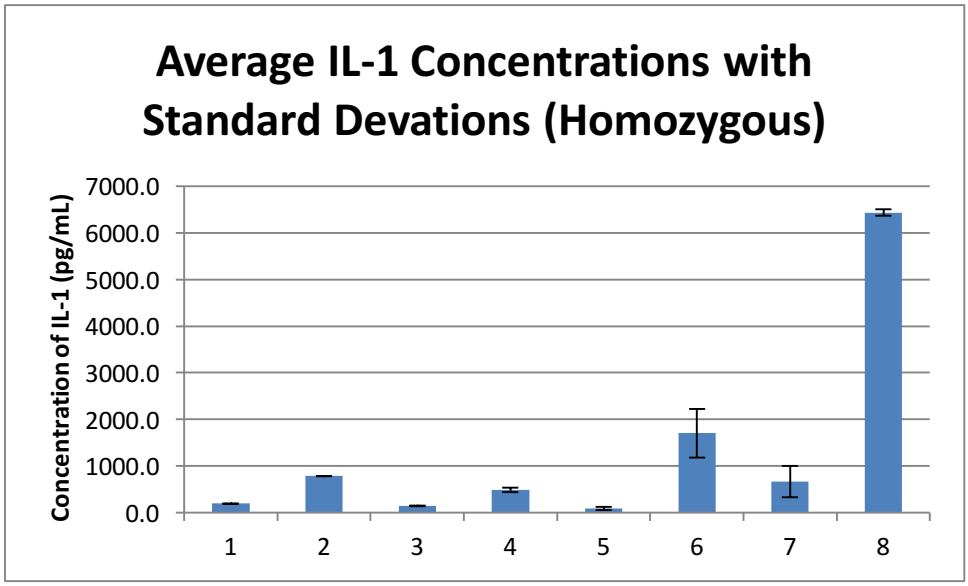


Figure 1: Average IL-1 Concentrations with Standard Deviations (Homozygous)
This figure shows the average concentrations of IL-1 in rats with the gene completely knocked-out. The standard deviation is shown using error bars.

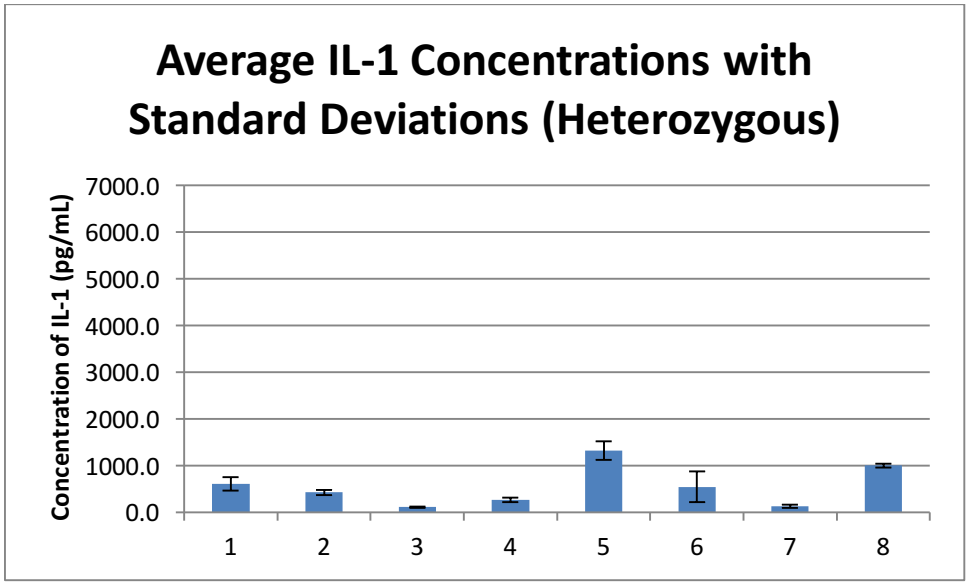


Figure 2: Average IL-1 Concentrations with Standard Deviations (Heterozygous)
This figure shows the average concentrations of IL-1 in rats with the gene partially knocked-out. The standard deviation is shown using error bars.

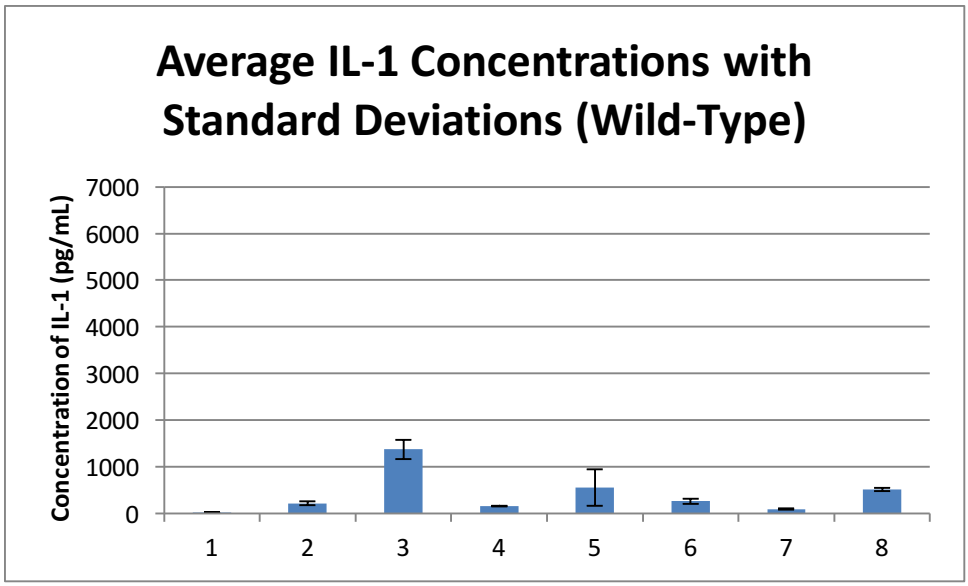


Figure 3: Average IL-1 Concentrations with Standard Deviations (Wild-Type)
This figure shows the concentrations of IL-1 in rats that have a fully-functional gene. The standard deviation is shown using error bars.

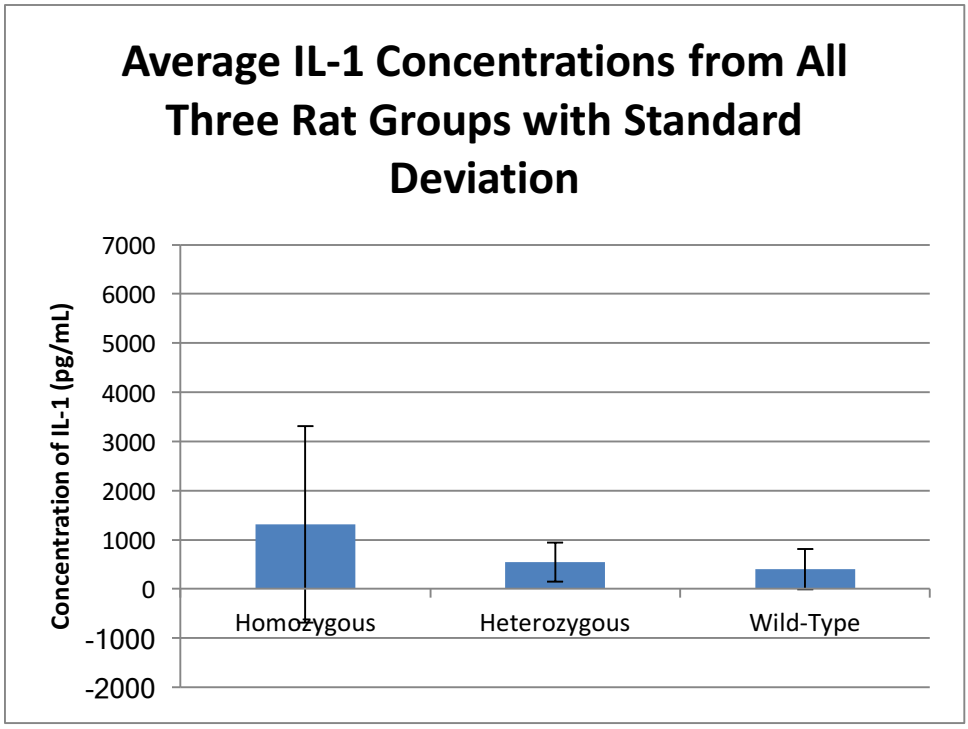


Figure 4: Average IL-1 Concentrations from All Three Rat Groups with Standard Deviations
This figure shows the average concentration of IL-1 in all three rat groups. The standard deviation is shown using error bars.

**b. TNF-α ELISA**

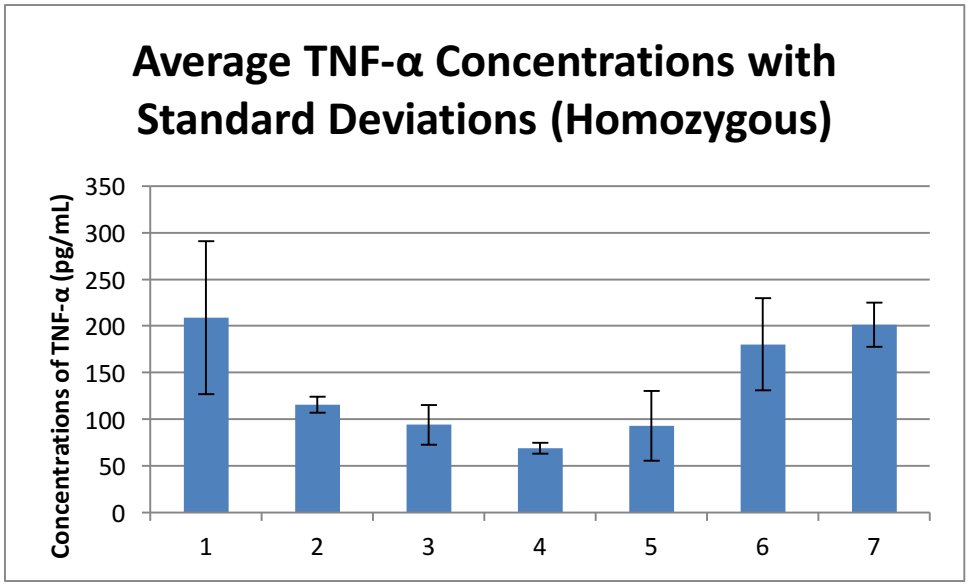


Figure 5: Average TNF-α Concentrations with Standard Deviations (Homozygous)
This figure shows the concentrations of TNF-α in rats with the gene completely knocked-out. The standard deviation is shown using error bars.

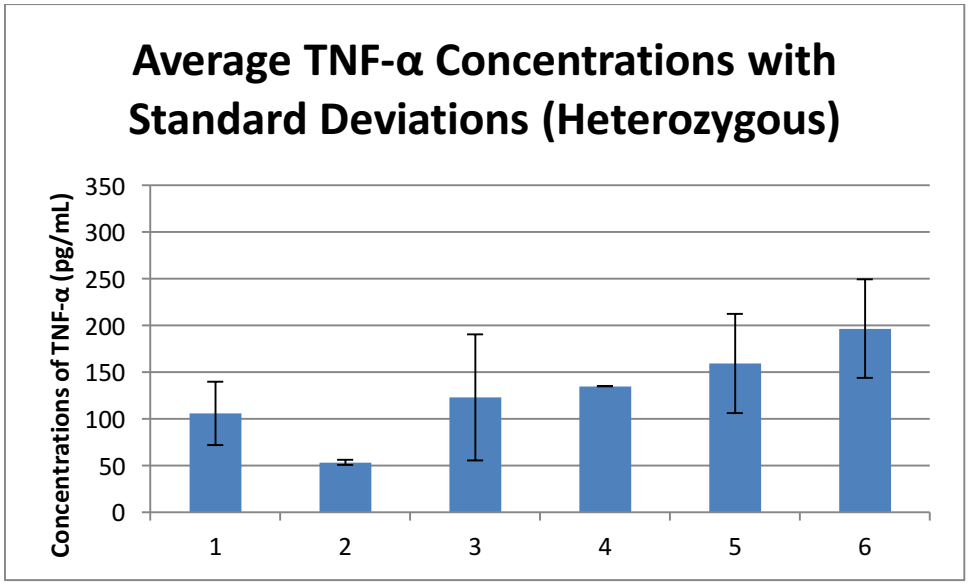


Figure 6: Average TNF-α Concentrations with Standard Deviations (Heterozygous)
This figure shows the concentrations of TNF-α in rats with the gene partially knocked-out. The standard deviation is shown using error bars.

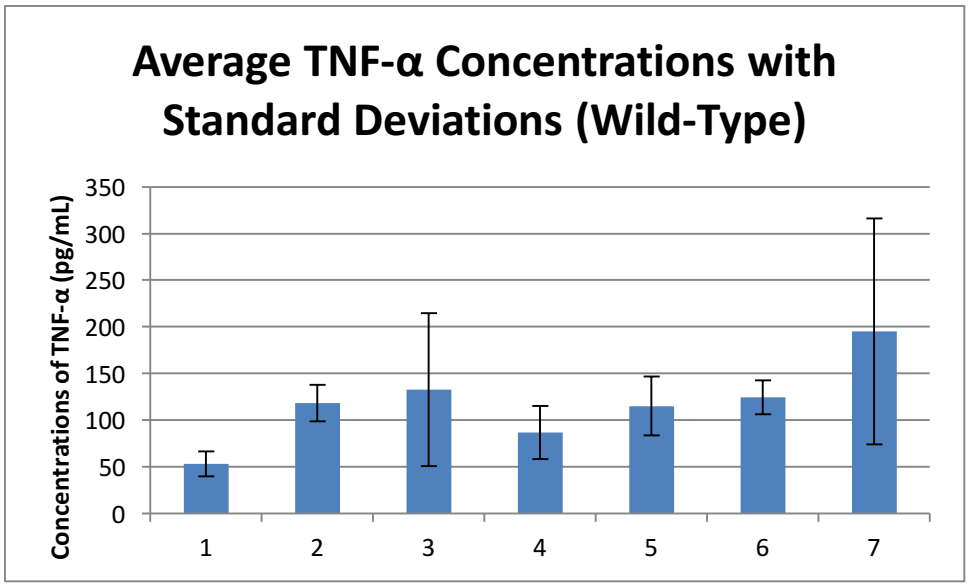


Figure 7: Average TNF-α Concentrations with Standard Deviations (Wild Type)
This figure shows the concentrations of TNF-α in rats with a fully functional gene. The standard deviation is shown using error bars.

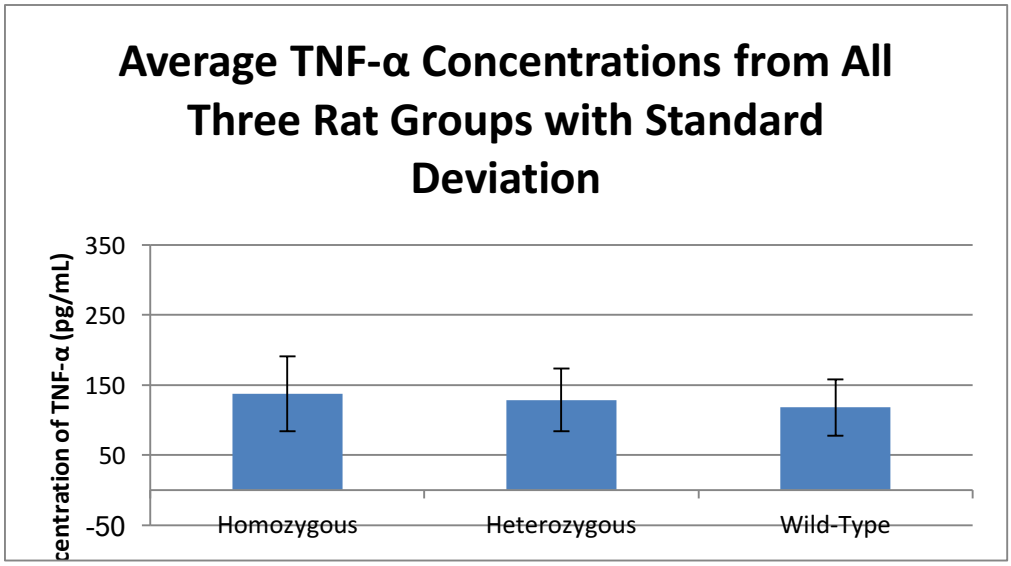


Figure 8: Average TNF-α Concentrations from All Three Rat Groups with Standard Deviations
This figure shows the average concentration of TNF-α in all three rat groups. The standard deviation is shown using error bars.

## Discussion

The results were not what I expected; however, they were interesting. My working hypothesis was that that there would be highly increased levels of inflammatory cytokines in pancreatic tissue from rats without either functional melanocortin receptor gene when compared to pancreatic tissue of normal rats and that there would be slightly increased levels of inflammatory cytokines in pancreatic tissues from rats with only one functional melanocortin receptor genes compared to pancreatic tissue of normal rats.

Statistical analysis of the results indicates that no significant differences in TNF-α or IL-1 levels were found in the pancreas of the rat groups ($p>0.05$). Therefore, the working hypothesis is rejected and the null hypothesis stating that there would be no difference in the levels of inflammatory cytokines among the three rat groups is accepted.

Previous research in the Beltz and Novak laboratories on the white adipose tissue and the blood from these rats also did not yield any statistically significant differences in the level of TNF-α between the three rat groups, suggesting that there is not a difference in levels of the inflammatory cytokines in the tissues investigated among the groups of this strain of rat.[2]

## Potential Topics for Future Research

There are several topics of research that could follow this project. One potential project is to repeat the IL-1 and TNF-α ELISAs using pancreatic tissues from high capacity runner (HCR) rats and comparing the levels to pancreatic

tissues with low capacity runner (LCR) rats. HCR rats are prone to being lean and have a higher capacity for exercise where LCR rats are prone to being obese and have a lower capacity for exercise. These strains of rats are outbred, meaning they are not as genetically similar as an inbred strain. Previous research in the Beltz and Novak laboratories on this strain of rat did yield statistically significant differences in the levels of cytokines in some tissues.

Another potential project is to repeat these ELISAs on different tissues in the melanocortin receptor knock-out rats. Levels of leptin circulating in the serum of these rats could be different, causing a difference in appetite.

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## Appendix A: Background

Obesity is a condition in which the weight of the body is more than 20% above a desirable standard as a result of excess adipose tissue.[5] Over a third of adults in the United States are obese.[5] Factors contributing to obesity include genetic factors, eating habits often taught early in life, overeating often to relieve tension, and social customs.[5] Rarely, there is trauma to or a tumor in the hypothalamus, which is the food-regulating center.[5] People who gain weight easily exhibit less nonexercise activity thermogenesis (NEAT) (See Appendix C for abbreviations), such as fidgeting, than people who do not.[5] Endothelial lipoprotein lipase is an enzyme that regulates triglyceride storage.[5] It is more active in abdominal fat than hip fat.[5] Treating obesity is difficult because most people who lose weight gain it back.[5] Treatment can include behavior modification programs, very-low-calorie diets, drugs, and surgery.[5] Treatment is important since obesity is a risk factor for cardiovascular disorders, diabetes, and high blood pressure.

### (a) Metabolic Syndrome

Metabolic syndrome is a collection of conditions that together lead to an increased chance of heart disease, stroke, and diabetes.[7] These conditions include increased blood pressure, high blood glucose, excess fat around the waist, and abnormal cholesterol or triglyceride levels.[7] Metabolic syndrome causes coronary heart disease (CHD) in which a plaque builds inside the coronary arteries.[8] Plaque hardening causes the arteries to narrow, reducing blood flow to

the heart muscles.[8] Other names for metabolic syndrome include dysmetabolic syndrome, hypertriglyceridemic waist, insulin resistance syndrome, obesity syndrome, and syndrome X.[8]

Metabolic syndrome is linked to obesity, inactivity, and insulin resistance.[7] There are other factors that increase the chances of having metabolic syndrome, including age, race, and other diseases.[7] As age increases, so does the chance of getting metabolic syndrome.[7] Mexican-Americans seem to have the greatest risk of getting metabolic syndrome.[7] Gestational diabetes or a family history of type II diabetes increases the risk of metabolic disease.[7] Cardiovascular disease, nonalcoholic fatty liver disease, and polycystic ovary syndrome are also risk factors for metabolic syndrome.[7]

Two other conditions often accompany metabolic syndrome: excessive blood clotting and low-grade inflammation in the body.[8] Gallstones and sleep apnea may also contribute to metabolic syndrome.[8] Metabolic syndrome may also be caused by side effects of medication that are often used to treat inflammation, allergies, HIV, and mental illnesses.[8] Women are more likely than men to have metabolic syndrome.[8] People who have metabolic syndrome usually have other signs of insulin insensitivity, including darkened skin on the back of the neck or underarms (acanthosis nigrican) or skin tags on the neck.[9] People with metabolic syndrome may also develop damage to the lining of arteries, a decrease in the kidneys' ability to remove salt from the blood, an

increase in triglyceride levels, higher risk of blood clot formation, and a decrease in insulin production.[9]

Metabolic syndrome is diagnosed if at least three of the following traits are present or medication is taken to control them: a waist circumference of at least 35 inches in women and 40 inches in men, at least 150 mg/dL of TAG found in the blood, less than 40 mg/dL of high-density lipoprotein (HDL) cholesterol in men and 50 mg/dL in women, a blood pressure of at least 130/85 mm Hg, or a fasting blood glucose of at least 100 mg/dL.[7] Excess fat around the waist is also known as the apple-shaped figure (See Appendix D).[7]

A fasting blood glucose level between 100 and 125 mg/dL is considered prediabetic, and a fasting blood glucose level of at least 126 mg/dL is considered diabetic.[8] Making heart-healthy lifestyle changes is first recommended to treat metabolic syndrome.[8] The goals of treatment are to reduce the risk of CHD and type II diabetes. Doctors can order a lipoprotein panel, which tests levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, and TAG to help diagnose metabolic syndrome.[8]

Treatment can include aggressive lifestyle changes and medication to help control blood pressure, cholesterol levels, and blood glucose.[7] Some life style changes include becoming physically active, losing weight, eating a diet that helps with hypertension, stopping smoking, and handling stress.[7]

b) **Inflammation**

Neutrophils are the most abundant cells in the early stages of inflammation.[4] When signaled by the mediators thrombin, histamine, and cytokines, such as IL-1 or TNF-α, vascular endothelial cells increase the number of E- and P-selectin molecules on their surface.[4] Neutrophils have molecules called mucins on their surface to bind to these selectins.[4] When the neutrophil tethers to the endothelial cells, it starts to roll in the direction of blood flow.[4] Chemokines and chemoattractants signal the neutrophil to trigger the G-protein-mediated activating signal, which leads to conformational change of the integrin adhesion molecules on the neutrophil.[4] These molecules now allow the neutrophil to stick to the surface of the blood vessel and eventually migrate transendothelially (through the endothelium).[4] The activating signal stimulates metabolic pathways to produce reactive oxygen intermediates and reactive nitrogen intermediates through a respiratory burst.[4] The release of these intermediates along with the release of mediators from the primary and secondary granules of the neutrophil kill many pathogens.[4] Neutrophils also phagocytize (eat) the pathogen. Dead cells and microorganisms, fluid, and several types of proteins make up pus.[4]

The characteristics of acute inflammation are swelling, redness, heat, pain, and loss of function (See Appendix E for comparisons of some common inflammatory cytokines).[4] This is caused by vasodilation, an increase in diameter of blood vessels.[4] Vascular permeability causes edema, an accumulation of fluid

in the tissue.[4] Histamine is a powerful mediator of inflammation that causes vasodilation and smooth muscle contraction.[4] Macrophages arrive in the tissue after neutrophils.[4]

Acute-phase response is characterized by induction of fever, increased synthesis of hormones, increased production of white blood cells (leukocytosis), and production of acute-phase proteins in the liver.[4] Many effects of the acute-phase response are due to IL-1, IL-6, and TNF-α.[4]

Pathogens that escape acute inflammation trigger chronic inflammation.[4] Chronic inflammation causes tissue damage and occurs in many autoimmune diseases and cancers.[4] Much of the resulting tissue wasting is caused by TNF-α.[4] Chronic inflammation is characterized by the accumulation of activated macrophages.[4] These macrophages continually release cytokines, stimulating the proliferation of fibroblasts and the production of collagen.[4] Scar tissue can develop at the site of inflammation through a process called fibrosis.[4] This reaction can interfere with the normal function of tissues.[4] Inflammation can also cause edema and formation of a granuloma, a tumor-like mass that has a central region of activated macrophages surrounded by activated lymphocytes.[4] Sometimes the macrophages fuse, forming a giant, multinucleated cell, or epithelioid cell.[4] Interferon alpha (IFN-α), produced by leukocytes to activate macrophages, increases transcription of the gene for TNF-α and stabilizes its messenger ribonucleic acid (mRNA).[4]

**c) Type II Diabetes (NIDDM)**

In NIDDM, at first pancreatic β cells hyperproliferate, but eventually apoptosis, or programmed cell death, occurs.[3] Metabolic syndrome precedes NIDDM.[6] Individuals with metabolic syndrome usually have abnormal clotting characteristics or inflammation and are predisposed to type II diabetes.[6] The lipid toxicity hypothesis states that full adipocytes release peroxisome proliferator-activated receptor gamma (PPARγ) from the surface of the cell.[6] PPARγ is the receptor which makes cells sensitive to insulin and making deposits of adipose in the cells.[6] Full adipose tissues release protein factors to attract macrophages, which come into the tissue from the blood and cause an inflammatory response.[6] This releases free fatty acids into the blood, which then travel to the liver and muscles. This leads to insulin insensitivity in these tissues.[6]

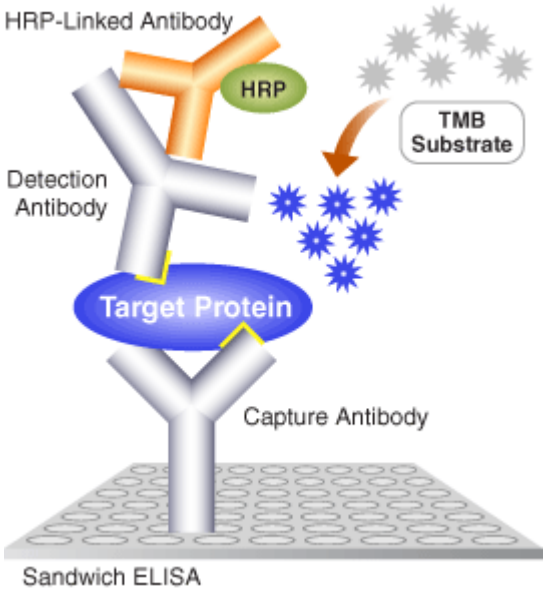
Type I diabetes (IDDM) and NIDDM have similar symptoms: excessive thirst, leading to polydipsia (excessive drinking); polyuria; glucosuria; and excessive yet incomplete breakdown of fatty acids in the liver, leading to ketoacidosis.[6] Ketoacidosis causes exhalation of acetone in the blood, causing a sweet breath odor, or to go to the brain, causing confusion.[6] NIDDM initially has milder symptoms.[6] The use of lipids as fuel may also cause lipid particles to be deposited on walls of blood vessels, causing several diseases, including cerebrovascular insufficiency, ischemic heart disease, peripheral vascular disease, and gangrene.[10] People suffering from diabetes mellitus also have polyphagia (excessive eating).[10] This polyphagia is due to increased hunger because of lack

of sugar in the cells.[11] Interestingly, weight loss also accompanies diabetes.[10] This is due to decreased ability of sugar to get into the cells. The cells then begin to use fatty acids for fuel. The lack of sugar in the cells also causes fatigue.[11] Diabetes may also cause blindness due to cataracts or damaged blood vessels of the retina.[10] Cataracts are caused by glucose on the lens leading to cloudiness.[10] NIDDM also makes sores heal slower and the diabetic becomes less resistant to infections.[11]

Complications of diabetes includes heart and blood vessel disease, neuropathy (nerve damage), nephropathy (kidney damage), eye damage, foot damage, hearing impairment, skin pathology, and Alzheimer's disease.[11] Digestion problems and erectile dysfunction are also possible concerns.[11] The nerve damage is caused by the excess sugar damaging the walls of capillaries that feed cells, such as neurons.[11] The damage to the capillaries also cause kidney damage and diabetic retinopathy (damage to the blood vessels of the retina).[11] Between nerve damage and poor circulation, diabetes can cause foot complications, which might require amputation.[11] A life-threatening complication is hyperglycemic hypersmolar nonketotic syndrome (HHNS).[11] This syndrome is characterized by a blood glucose level higher than 600 mg/dL (33.3 mmol/L), dry mouth, extreme thirst, fever more than 101°F, drowsiness, confusion, vision loss, hallucinations, and dark urine.[11] This is caused by extremely high blood sugar levels that turn the blood thick.[11]

Tests for diabetes include the glucose-tolerance test, in which the individual fasts overnight and drinks a glass of water with 100g of dissolved glucose in the morning.[6] The blood glucose is tested before the sugar water and at 30 minute intervals thereafter.[6] Healthy individuals will not have a spike in blood sugar greater than 10 mM and little to no glucose is shed in the urine.[6] Another test for NIDDM is the glycated hemoglobin (A1C) test.[11] This indicates the average blood glucose for at most three months.[11] It measures the percentage of blood glucose attached to hemoglobin, a protein that carries oxygen in red blood cells.[11] More glucose in the blood means that more hemoglobin will have sugar attached to it.[11]

**Appendix B: Sandwich ELISA**




http://www.ebioworld.com/2012/01/elisa-enzyme-linked-immunosorbent-assay_3222.html

**Appendix C: Abbreviations**

| | |
|---|---|
| A1C | (Glycated Hemoglobin) |
| BSA | (Bovine Serum Albumin) |
| CHD | (Coronary Heart Disease) |
| ELISA | (Enzyme-Linked Immunosorbent Assay) |
| ER | (Endoplasmic Reticulum) |
| GI | (Gastrointestinal) |
| GIP | (Glucose-dependent Insulinotropic Peptide) |
| HCR | (High Capacity Runner) |
| HDL | (High-Density Lipoprotein) |
| hGH | (Human Growth Hormone) |
| HHNS | (Hyperglycemic Hypersmolar Nonketotic Syndrome) |
| HIV | (Human Immunodeficiency Virus) |
| IDDM | (Type I Diabetes) |
| IFN-α | (Interferon Alpha) |
| IL-1 | (Interleukin-1) |
| IRS-1 | (Insulin Receptor Substrate 1) |
| LCR | (Low Capacity Runner) |
| LDL | (Low-Density Lipoprotein) |
| mRNA | (Messenger Ribonucleic Acid) |
| NEAT | (Nonexercise Activity Thermogenesis) |
| NIDDM | (Type II Diabetes) |

| | |
|---|---|
| PPARγ | (Peroxisome Proliferator-Activated Receptor Gamma) |
| RTK | (Receptor Tyrosine Kinase) |
| TAG | (Triglyceride) |
| TBS | (Tris Buffer Saline) |
| TLR | (Toll receptors) |
| TNF-α | (Tumor Necrosis Factor-Alpha) |

**Appendix D: Apple and Pear Figures**

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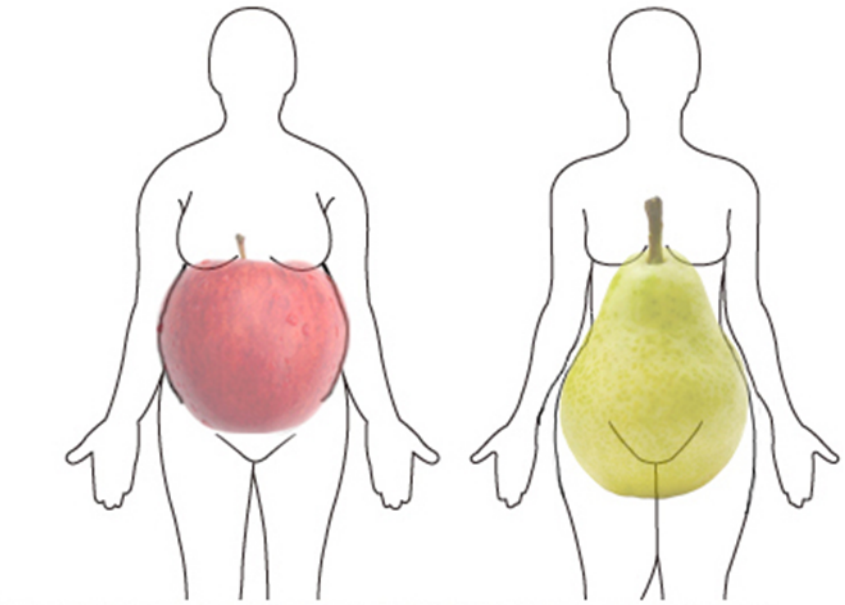

**Appendix E: Comparisons of Some Common Inflammatory Cytokines**

| Effect | IL-1 | TNF-α | IL-6 |
|---|---|---|---|
| **Pyrogenic (induces fever)** | + | + | + |
| **Synthesis of acute-phase proteins by liver** | + | + | + |
| **Increased vascular permeability** | + | + | + |
| **Increased adhesion molecules on vascular endothelium** | + | + | - |
| **Fibroblast proliferation** | + | + | - |
| **Platelet production** | + | - | + |
| **Chemokine induction** | + | + | - |
| **T-cell activation** | + | + | + |
| **B-cell activation** | + | + | + |
| **Increased immunoglobulin synthesis** | - | - | + |

4