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The Impact of Food Deprivation on Cardiovascular Physiology

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THE IMPACT OF FOOD DEPRIVATION ON CARDIOVASCULAR PHYSIOLOGY

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Dedication

The last four years have been a lot. There have been some high high's and some low low's. And now the end of this phase is near, and I am transitioning to the next phase. As I close out on this phase I offer the following dedication.

I dedicate this dissertation to my family, for it is for my family, primarily, that I have done this dissertation.

My beautiful wife, Adrienne. We have been through so much together, and I find your love and support to be invaluable. Thank you for being there for me and for believing in me. I want to be the best version of myself for you. I love you so very much.

My beautiful children, Evelyn and Louetta. You two are shining lights in my life. It is one of the great joys in my life to be your father. I also find yall's love and support to be invaluable, and I also want to be the best version of myself for yall. I love yall so very much.

My beautiful extended family. Grandparents, great aunts, great uncles, parents, aunts, uncles, siblings, cousins, in-laws, and the list goes on. My life is full of family that I feel so loved and supported by. This is truly one of the greatest blessings in my life. I would not be the person that I am today if it were not for the love and support of my family. I want yall to feel my love and support for yall, and I love yall so very much.

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Definitions and Abbreviations

Below is a list of important terms, and abbreviations utilized throughout this dissertation.

- Acute Food Deprivation = a stand-alone, continual period of no food consumption.
- Chronic Food Deprivation = a regimen that cycles between a period of acute food deprivation and a period of normal food consumption.
- Cardiovascular Physiology = the functioning of the system that facilitates the circulation of blood throughout the body.

- ABPM = ambulatory blood pressure monitor
- ACSM = American College of Sports Medicine
- ACTH = adrenocorticotropic hormone
- ANOVA = analysis of variance

- BP = blood pressure
- BRS = baroreflex sensitivity
- BTBBP = beat-to-beat blood pressure

- COVID-19 = coronavirus disease 2019
- CRH = corticotropic releasing hormone

- DAP = diastolic arterial pressure
- DBP = diastolic blood pressure
- DEXA = dual-energy x-ray absorptiometry

- EDTA = ethylenediaminetetraacetic acid
- ELISA = enzyme-linked immunosorbent assay
- EKG = electrocardiography

- HF = high frequency
- HR = heart rate
- HRV = heart rate variability

- iNOS = inducible nitric oxide synthase

- LEAP2 = liver-expressed antimicrobial peptide 2
- LF = low frequency
- LF/HF = low frequency to high frequency ratio

- MSNA = muscle sympathetic nerve activity

- nNOS = neuronal nitric oxide synthase
- NOS = nitric oxide synthase
- NPY = neuro-peptide Y

- RNA = ribonucleic acid
- RR = respiration rate
- RRI = R-to-R interval

- SAP = systolic arterial pressure
- SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
- SBP = systolic blood pressure

Abstract

Introduction: Despite the commonality of fasting, there still remains a lack of scientific research, specifically in regard to the impact of fasting on cardiovascular physiology. Thus, the goal of this research project was to further explore the relationship between fasting and cardiovascular physiology. **Hypothesis:** The hypothesis of this research project was that a 24-hr fast would likely lead to increased BP and HR, along with blood biomarker changes, and that experiencing this 24-hr fast twice a week would lead to reduced BP and HR along with altered circulating blood biomarker levels. **Methods:** In order to test these hypotheses, cardiovascular and blood biomarker factors were assessed both before, during and after a 24-hr fast, and throughout a 4-wk period of two 24-hr fasts per week. **Results:** After the 24-hr fast there was increased resting BP (SBP, p=0.062; DBP, p=0.101) and HR (p=0.125), in addition to decreased overall average ambulatory BP (SBP, p=0.159; DBP, p=0.167) and HR (p=0.076) throughout the 24-hr fast. Blood glucose (p=0.012) and plasma NPY (p=0.007) were decreased, and plasma ghrelin (p=0.171) and plasma LEAP2 (p=0.203) were increased after the 24-hr fast. Resting BP (SBP, p=0.004; DBP, p=0.202) was decreased, and autonomic function showed a shift toward lessened sympathetic activity (↑ RRI, p=0.125; ↓ RRI-LF/HF ratio, p=0.293) at the end of the 4 weeks of fasting, and the decrease in BP was seen as early as 2 weeks of fasting. Plasma ghrelin (p=0.372) was increased at the end of the 4 weeks of fasting with little to no change in blood glucose (p=1.000), plasma LEAP2 (p=1.000) and plasma NPY (p=1.000). Plasma LEAP2 (p=0.693) and plasma NPY (p=0.473) did decrease after 2 weeks of fasting before returning to approximately baseline levels after 4 weeks of

fasting. The cardiovascular changes from 24 hours of fasting were most correlated to blood glucose and plasma ghrelin, and the changes from 4 weeks of fasting were most correlated to plasma ghrelin and plasma NPY. **Discussion:** It appears that fasting may have a mild impact on cardiovascular physiology; both during a 24-hr fast and as an adaptation to 4 weeks of fasting.

1 Introduction

Food deprivation, commonly known as fasting, is a period of time where food is not consumed. For humans, it is a globally ubiquitous practice, both voluntarily and involuntarily. Voluntarily, food deprivation commonly occurs as part of religious practice, health practice, and social protest. Involuntarily, food deprivation commonly occurs due to famine, war, poverty, and slavery. Generally, food deprivation can be organized into two broad categories, acute and chronic. Acute food deprivation is a one-time, stand-alone, continual period of no food consumption. The period of acute food deprivation could last from only a few minutes or up to multiple months. On the other hand, chronic food deprivation, also known as intermittent fasting, is a regimen that cycles between a period of acute food deprivation and a period of normal food consumption. Chronic food deprivation could mean participating in a bout of acute food deprivation as little as once a month or up to as frequently as once every day. Typically, a bout of acute food deprivation, whether stand-alone or as part of chronic food deprivation, lasts longer than the typical time between meals.

Although the practice of food deprivation is rather ubiquitous and timeless, the current state of research in the field surrounding food deprivation is informative but limited in scope. Additionally, the current research is broadly diverse in experimental design, likely due to the broad possibilities of experimental design for both acute and chronic food deprivation. Research can be found with an acute food deprivation protocol that lasts less than 24 hours, and with an acute protocol that lasts more than 40 days, and various times in-between. It is also possible to find research with chronic food deprivation protocols

that differ in regard to the length and frequency of bouts of acute food deprivation in addition to the total length of the chronic food deprivation regimen. Additionally, there are protocols of bouts of acute food deprivation within chronic food deprivation regimens where the acute food deprivation is not a total cessation of food intake, but instead a decreased caloric amount of food intake (i.e. caloric restriction). Moreover, there is also variety in the focus of food deprivation research, with a large amount of existing research focusing on body mass management through food deprivation. The goal of this dissertation was to explore some of the current limitations in the research surrounding food deprivation with an experimental protocol that linked both acute and chronic food deprivation while trying to understand how food deprivation affects cardiovascular physiology.

Broadly, cardiovascular physiology entails the functioning of the system that circulates blood throughout the body. The cardiovascular system is primarily comprised of the heart and blood vessels, and is also affected by other body systems. The autonomic nervous system is of particular note as it directly affects the cardiovascular system through sympathetic (broadly an excitatory effect; i.e. increased heart rate) and parasympathetic (broadly an inhibitory effect; i.e. decreased heart rate) action. Additionally, the cardiovascular system can be impacted by a variety of experiences, conditions, and environments, such as food deprivation.

The current state of research regarding the impact of food deprivation on cardiovascular physiology shows that, in general, a bout of acute food deprivation appears to be a sympatho-excitatory stressor. In 2007, Chan et al found that healthy women experienced

a decrease in heart rate variability after 72 hours of food deprivation, which is commonly associated with increased sympathetic activity (Chan, Mietus, Raciti, Goldberger, & Mantzoros, 2007). Then, in 2012, Herbert et al showed that 24 hours of food deprivation led to an increase in heart rate in healthy women (Herbert et al., 2012). In 2015, Schulz et al found that 18 hours of food deprivation in healthy women led to increased amplitude of heartbeat evoked potentials in the brain (Schulz et al., 2015). In addition, Seker et al (2017), studying normotensive humans participating in Ramadan, recorded an increase in systolic blood pressure after 17 hours of food deprivation (Seker et al., 2017). These studies suggest that a bout of acute food deprivation is generally a sympatho-excitatory cardiovascular stressor.

However, it is also important to note that while the majority of food deprivation studies show an excitatory effect of acute food deprivation on cardiovascular physiology, there are a few studies that have showed a different response to acute food deprivation. Namely, one study by Solianik et al found participants to have decreased heart rate and blood pressure in addition to increased heart rate variability, after a 48-hour fast (Solianik, Sujeta, Terentjeviene, & Skurvydas, 2016). This difference of response suggests that there is nuance to how the cardiovascular system responds to food deprivation.

If acute food deprivation is a sympatho-excitatory stressor, then perhaps regularly experiencing acute food deprivation would lead to a reduced basal sympathetic activity level adaptation. Current research regarding chronic food deprivation lends some support to this notion. Epidemiological studies suggest that persons who have participated in

some form of a chronic food deprivation regimen for a long period of time have a reduced body mass, increased life span, and reduced risk of cardiovascular and metabolic disease compared to those who do not regularly fast (Rizza, Veronese, & Fontana, 2014; Stockman, Thomas, Burke, & Apovian, 2018; Tinsley & La Bounty, 2015; Varady & Hellerstein, 2007). In terms of experimental evidence, Harvie et al found that overweight women who participated in a day of 25% caloric restriction twice per week for one month experienced a reduction in blood pressure (Harvie et al., 2011). And Samad et al found that healthy adults participating in Ramadan experienced a reduction in blood pressure throughout Ramadan (Samad et al., 2015). However, Heilbronn et al (2005) showed no changes in blood pressure after three weeks of alternate day fasting in healthy humans (Heilbronn, Smith, Martin, Anton, & Ravussin, 2005). These studies support the notion that chronic food deprivation leads to a reduced basal sympathetic activity adaptation, but this adaptation may possibly be connected to the type and length of the chronic food deprivation regimen.

As to why the cessation of food intake leads to an excitatory effect on cardiovascular physiology, it is possibly occurring as a chain of events starting with a decrease in blood glucose which elicits a change in circulating hormone levels. The existing research shows that an acute food deprivation bout of 24 hours leads to a decrease in blood glucose (Merimee & Tyson, 1974). Furthermore, Young et al found an increase in urine epinephrine levels after 48 hours of food deprivation in men (J. B. Young, Rosa, & Landsberg, 1984). Additionally, Espelund et al noted that a deprivation of food for 24 hours led to an increase in circulating ghrelin levels in healthy humans (Espelund et al.,

2005). These studies provide evidence that acute food deprivation causes a change in blood glucose and circulating hormones, which also have a link to autonomic nervous system activity, and thus could be contributing to the change in sympathetic activity.

1.1 Research Focus Opportunity

While the current research is largely supportive of the notion that acute food deprivation is a sympatho-excitatory stressor, and that chronic food deprivation leads to a reduced basal sympathetic activity adaptation, the current research is not definitive and is limited in volume and broad in participant population and experimental design.

Therefore, this dissertation was designed to:

1) Build upon the existing knowledge in the field,
2) Create an experimental protocol that linked both acute and chronic food deprivation, and
3) Utilize an acute and chronic experimental protocol that was a sufficient stressor to cause an effect.

The following hypothesis and specific aims guided this dissertation.

1.2 Hypothesis

The two-part hypothesis of this dissertation was:

1) It was hypothesized that a 24-hour bout of acute food deprivation would likely lead to an excitatory cardiovascular response in healthy humans, associated with hormonal changes. It was expected that blood pressure and heart rate would be increased after 24 hours of acute food deprivation, along with decreased blood glucose, increased plasma ghrelin, increased plasma NPY, and decreased plasma LEAP2.

2) It was further hypothesized that experiencing this 24-hour bout of acute food deprivation twice per week for 4 weeks would lead to cardiovascular adaptations stemming from reduced basal sympathetic tone and altered circulating hormone levels. It was expected that blood pressure and heart rate would be decreased after 4 weeks of chronic food deprivation, along with increased heart rate variability, decreased plasma ghrelin, decreased plasma NPY, and increased plasma LEAP2.

1.3 Specific Aims

Specific aim one was to evaluate the hemodynamic and molecular changes, related to cardiovascular physiology, that occurred within and following a 24-hour acute period of food deprivation. The primary outcome variables for specific aim one were: blood pressure, heart rate, and blood biomarkers.

Specific aim two was to evaluate the autonomic, hemodynamic, and molecular changes, related to cardiovascular physiology, that occurred during and following a 4-week period of a chronic food deprivation regimen. The primary outcome variables for specific aim

two were: blood pressure, heart rate (to include heart rate variability), and blood biomarkers.

2 Review of Literature

The previous chapter served as an introduction to the dissertation research project, and this chapter serves to delve into a detailed review of the field of research surrounding the impact of food deprivation on cardiovascular physiology.

2.1 History of Food Deprivation Research

The practice of food deprivation has been a part of human life since time immemorial, largely seen as a component of religious practice and asceticism (Arbesmann, 1949). Food deprivation as a topic of research has been noted in publications since at least the early 1600's (Hildanus, 1646) and is commonly written about under the following monikers: fasting, food deprivation, and caloric restriction. These early accounts, up through the 1800's (Dougal, 1881; Granger, 1809; Hildanus, 1646; Mackenzie, 1776), largely focused on case study medical descriptions of individuals purportedly fasting for up to multiple years. Then in the very late 1800's, and going forward, publications started appearing that explored the effects of food deprivation with controlled trials (Hoover & Sollmann, 1897).

A thorough search of the literature on PubMed in October of 2019 showed that research publications related to food deprivation remained relatively low until the late 1940's, before increasing, but still largely remained below 50 publications a year. Then, during the 1960's, research publications further increased to around 150 – 200 publications a year, and remained in this range through the end of the twentieth century. The research concerning food deprivation in the latter half of the 1900's was broad in scope and

design, but seemed to primarily center under the umbrella of metabolism related topics such as lipid and glucose concentrations in the blood, obesity, and diabetes.

Since the year 2000 there was another further increase in the number of research publications in the realm of food deprivation, and in the last few years there have been around 600 publications a year. Similar to the latter half of the 1900's, the 2000's thus far have produced research that is largely centered under the umbrella of metabolism related topics. Therefore, at the end of the 2010's the current state of the field with regard to food deprivation research remains broad in experimental design, but limited in scope, even though there have been around 14,100 publications in the last almost 375 years.

Furthermore, refining the original PubMed search of the 14,100 food deprivation research publications, only around 6% were related to cardiovascular physiology. This 6% (~900) of publications extends from the mid 1900's until present day, and largely remained under 10 publications a year until the twenty first century, where it increased, and has been around 50 publications a year in the last few years. Further scrutiny of these ~900 publications reveals that 140 directly relate to the effect of food deprivation on cardiovascular physiology and/or cardiovascular morphology. And delving into these 140 publications shows that ~46% (65) of them focused on human participants, with 75% of that 46% focusing on "non-normal" (ex. disease conditions) participants.

All of this historical information indicates that in the last 375 years of published research on food deprivation there are only around 17 published research articles that focus on the

basic science of the impact of food deprivation on cardiovascular physiology in healthy, "normal" human participants.

2.2 Basic Physiology of Food Deprivation

Globally, humans produce a 23% surplus of food, yet 10.8% of all humans are undernourished; additionally, in all but one continent on Earth, obesity occurs at a higher rate than undernourishment (Gould, 2017). This information seems to suggest that most people (pending factors such as access and availability) have access to an abundance of food (i.e. food security).

When a person consumes food, it passes first into the stomach via the esophagus, after it is chewed and swallowed. After leaving the stomach it passes through the small intestine and then the colon, before what remains is evacuated from the body. The stomach functions largely to break down food in preparation for nutrient absorption, and to modulate hormonal and neural, enteric signaling (Muller, Canfora, & Blaak, 2018). Nutrient absorption from the recently broken-down food primarily occurs within the small intestine, with the colon being primarily responsible for absorbing water and electrolytes in addition to storing fecal matter prior to its evacuation (Muller et al., 2018).

Research shows that adults experiencing food security will typically wait 4 to 7 hours between eating meals while they are awake, and that they will wait around 12 hours from supper to breakfast, which incorporates the overnight sleeping period (Ishihara et al., 1985). Regarding food transit time in adults, research has shown that it only takes a few

seconds after swallowing for food to arrive in the stomach (Cordova-Fraga et al., 2008), and it takes around 3.6 to 5.6 hours for the stomach to empty after consuming a meal (Sugita, Matsumoto, Tsukano, Fukunaga, & Yamamoto, 2019). Additionally, the average and median small intestine transit time is around 3.3 hours (Fischer & Fadda, 2016; Lawrence, Crison, & Amidon, 1996).

Overall, the total digestive system transit time for adults, meaning ingestion to defecation, lasts around 34 to 56 hours (Kelsay, Behall, & Prather, 1978), but the previous points indicate that the vast majority of the body's nutrient receival occurs within the first 9 hours (from mouth entry through small intestine transit time). Taken together, this evidence seems to indicate that awake adults under food secure conditions will experience physiological and psychological food satiety for up to around 7 hours after eating a meal. Therefore, it could be surmised that it is not until the food deprivation period exceeds this initial 7 hours that food deprivation begins to become an abnormal stressor.

It is also important to note that there are a variety of psychological and physiological factors surrounding food consumption, and that these factors play a role in both the pre and post prandial periods. The psychological factors can be quite complex and do not necessarily correlate with physiological satiety (van der Laan, de Ridder, Viergever, & Smeets, 2011); these psychological factors will not be discussed in this review of literature. This review of literature will instead focus on the physiological factors, such as blood glucose and appetite related hormones, which are also complex, and also connect to the overall physiological functioning of the body in a variety of areas.

2.2.1 Glucose

Glucose is a monosaccharide and the primary carbohydrate fuel source for metabolism throughout the body. When carbohydrates are consumed they are broken down to glucose, which can then be absorbed into the blood stream and circulated throughout the body for utilization and storage (Chen et al., 2019). At the conclusion of a meal, glucose levels in the blood will generally rise above baseline for around 1 hour, and then fall back to baseline levels during the next hour (Jarrett, Baker, Keen, & Oakley, 1972). This two-hour rise and fall of blood glucose levels is utilized during oral glucose tolerance tests to assess glucose tolerance (Nelson, 1988). A study evaluating participants over a 24-hour period with normal eating found this prototypical rise and fall in blood glucose levels occurring at each meal (Christensen, Hansen, Weeke, & Lundbaek, 1978).

After consuming a meal, blood glucose levels will continue to drop, after this initial 1-hour rise, until around 48 hours post prandial, where they will then remain stable for at least 72 hours post prandial (Hojlund et al., 2001; Merimee & Tyson, 1974). Additionally, a study consisting of 5 days (120 hours) of food and water deprivation found blood glucose levels to decrease to a low point after 3 days (72 hours), and then rise back to just under baseline levels during the remaining 2 days (Papagiannopoulos, Sideris, Boschmann, Koutsoni, & Dotsika, 2013). Furthermore, another study that had participants fast for 42 days showed a similar trend for blood glucose where it decreased to a low point after 3 days, and then increased slightly for the next 4 days before maintaining at a relatively stable level until the end of the 42 day fast (Marliss, Aoki, Unger, Soeldner, & Cahill, 1970).

During these studies the lowest point of blood glucose levels (~3.3 mmol/L) was generally found to just cross the threshold for the American Diabetes Association's (ADA) definition of hypoglycemia (< 3.9 mmol/L) (Iqbal & Heller, 2016), starting somewhere in the 24 to 36 hours post prandial period, and in most studies blood glucose returned to above this hypoglycemia zone. It is also important to note that while the ADA ascribes a number value to hypoglycemia, it also states that the point of reaching hypoglycemia is particular to an individual with the important marker of hypoglycemia being the initiation of physical symptoms such as: anxiety, irritability, lightheadedness, nausea, weakness, headache, etc. Of the aforementioned fasting studies, only one mentioned their participants occasionally experiencing negative physical symptoms, and this study was the only one that employed both food and water fasting. Additionally, this study noted "a satisfactory physical condition of the participants during all 5 days" (Papagiannopoulos et al., 2013). Therefore, it appears that the alterations in blood glucose during fasting in healthy individuals largely do not involve hypoglycemia.

2.2.2 Appetite Related Hormones

In addition to glucose there are several appetite related hormones that play a physiological role in food consumption. Some of the prominent appetite related hormones are insulin, glucagon, ghrelin, and cortisol.

2.2.2.1 Insulin & Glucagon

Insulin is a 51 amino acid, two-chain monomer that is released from beta cells of the pancreas, and is primarily associated with blood glucose management (De Meyts, 2004). In a study evaluating participants over a 24-hour period with normal eating, blood insulin levels increased with each increase in blood glucose levels occurring at each meal (Christensen et al., 1978). Furthermore, another study evaluating the response to an oral glucose tolerance test found that blood insulin increased and decreased in tandem with alterations in blood glucose (Broglio et al., 2004). Also in this study, when insulin was administered during normoglycemia there was a decrease in blood glucose levels (Broglio et al., 2004). During food consumption, insulin levels in the blood tend to rise dramatically, from a low pre-prandial point (Ott et al., 2012), and then following the cessation of food consumption, insulin levels in the blood tend to decrease linearly from the food consumption high point throughout a 72-hour fast. This decrease of insulin levels during food deprivation is a steep decrease for the first 12 hours of fasting and a gradual decrease for the remaining time (Hojlund et al., 2001). A 42-day fast showed a similar pattern where blood insulin levels decreased steeply for the first 5 days, and then increased very slightly until day 14 before decreasing very gradually until day 42 (Marliss et al., 1970).

Glucagon is a 29 amino acid polypeptide that is secreted by alpha cells of the pancreas, and, like insulin, is primarily associated with blood glucose management (Downes, 2003). Throughout a 24-hour period of normal food consumption, blood glucagon levels remain relatively constant with little to no change (Christensen et al., 1978).

Additionally, a study found that during an oral glucose tolerance test, blood glucagon levels remained unchanged (Broglio et al., 2004). Also in this study, when glucagon was administered during normoglycemia there was an increase in blood glucose levels (Broglio et al., 2004). Furthermore, a study investigating glucagon's response to alterations in blood glucose found that blood glucagon levels increased during induced hypoglycemia and decreased during induced hyperglycemia (Gerich et al., 1974). During fasting, glucagon tends to follow an inverse linear pattern to that of insulin, where glucagon levels in the blood increase linearly throughout a 72-hour fast at a moderate rate (Hojlund et al., 2001).

These findings seem to indicate that insulin and glucagon work together to manage blood glucose levels, with insulin levels fluctuating more. In healthy, non-fasting and fasting individuals, blood glucose seems to either not enter into hypoglycemia or just exists in the outer bounds of normoglycemia.

In contrast with insulin and glucagon, cortisol and ghrelin tend to follow an oppositional, cyclical pattern during fasting.

2.2.2.2 Cortisol & Ghrelin

Cortisol is a steroid hormone (glucocorticoid) that is released from the adrenal cortex, and is primarily associated with stress and blood glucose management (Thau & Sharma, 2019). During normal food consumption cortisol tends to follow a cyclical pattern where it increases during the sleeping period to a high point just after breakfast, and then decreases during the awake period to a low point in the first third of the sleeping period;

blood cortisol levels also show small increase spikes immediately following a meal (Bhake et al., 2019). Throughout a 72-hour fast, blood cortisol levels follow a similar cyclical pattern to normal food consumption, rising at night and falling during the day (Espelund et al., 2005). Additionally, the 24-hour average for each 24-hour blood cortisol cycle increases slightly throughout a 72-hour fast (Espelund et al., 2005).

Ghrelin is a 28 amino acid hormone that is predominantly secreted by cells of the stomach, and is primarily associated with hunger (Perchard & Clayton, 2017). Ghrelin, during normal food consumption, will increase while no food is being consumed to a high point just as food consumption begins, and then will decrease for 2 to 4 hours before increasing again until food is consumed once again (Shiiya et al., 2002). This fall and rise of ghrelin seems to correlate to the gastric emptying time, as mentioned previously (Sugita et al., 2019). Similarly, blood ghrelin levels have been shown to decrease as both blood glucose levels increase during a glucose tolerance test, and as blood insulin levels increase during an insulin tolerance test (Broglio et al., 2004). However, research has also shown that blood ghrelin levels do not decrease or have a markedly blunted reaction in response to glucagon (which increases blood glucose), arginine (which increases blood insulin), a bolus of essential amino acids, or a primarily lipid beverage (Broglio et al., 2004; Foster-Schubert et al., 2008; Knerr, Groschl, Rascher, & Rauh, 2003). During a 72-hour fast, ghrelin will show a 24-hour cyclical pattern where blood ghrelin levels will rise during the day and fall during the night; this is directly oppositional to the pattern of blood cortisol levels (Espelund et al., 2005). Furthermore, the 24-hour average for each

blood ghrelin 24-hour cycle decreases slightly throughout a 72-hour fast (Espelund et al., 2005).

Although cortisol and ghrelin show an oppositional, cyclical relationship throughout a 24-hour period, the oppositionality of this relationship appears to hinge upon the removal of the involvement of food intake. This seems to be due to the observation that while cortisol and ghrelin both appear to respond to food intake, they each respond with opposite changes, and ghrelin appears to be more singularly influenced by food intake, specifically with regard to changes in glucose and insulin.

2.2.3 Other Hormones (NPY & LEAP2)

In addition to insulin, glucagon, cortisol, and ghrelin there are two other hormones that may play an important role in food deprivation physiology, however there is currently limited scientific evidence relating these two hormones to food deprivation, especially in humans.

The first hormone is neuro-peptide Y (NPY), which is a 36-residue peptide that was first isolated from the brain of pigs, and first described in the literature in 1982 (Tatemoto, 1982; Tatemoto, Carlquist, & Mutt, 1982). NPY is expressed within neurons throughout the brain, gut, and adrenals, and it is understood to be the most abundant neuropeptide in the brain (Holzer, Reichmann, & Farzi, 2012). There are several basic neuronal physiological functions for which NPY is related (Malva et al., 2012). Of particular importance to this review of literature is NPY's connection to food consumption (Woods et al., 1998). Rats that were given an intracerebroventricular injection of NPY showed an

increase in food intake, and during times when eating would normally not occur (Levine & Morley, 1984). Furthermore, rats that were deprived of food for 48 hours showed an increase in NPY RNA levels in various areas of the brain, compared to food ad libitum controls (Bi, Robinson, & Moran, 2003). Another study showed this same increase phenomenon with regard to NPY concentrations in the brain during a 48-hour fast, with the additional finding that brain NPY levels decreased to control levels after refeeding (Yoshihara, Honma, Katsuno, & Honma, 1996). In addition to changes in the brain, a study found an increase in NPY levels in the adrenals of mice after 24 hours of food deprivation, and that the increase in adrenal NPY levels after the 24-hour fast was necessary to cause fasting induced increases in urine epinephrine levels, and to maintain fasted state euglycemia (M. Wang, Wang, & Whim, 2016). Additional research in rodents has shown that hypothalamic NPY neurons are directly activated by ghrelin (Hashiguchi et al., 2017), and directly inhibited by insulin (Belgardt, Okamura, & Bruning, 2009) and glucose (Burdakov, Luckman, & Verkhratsky, 2005).

In humans, NPY levels have been measured in cerebrospinal fluid (CSF) and blood, with NPY levels in the CSF being approximately 2-fold higher (Brunani et al., 1995). There is limited information regarding the circadian rhythm values of NPY in humans. One study that measured blood NPY levels throughout a 24-hour period in humans who consumed food normally, found that blood NPY levels fluctuated cyclically with high peaks right at or right before meals, and low points between meals, with a maximum fluctuation range of 90 pg/ml (Galusca et al., 2015). Another study measuring blood NPY levels

throughout a 24-hour period of normal food consumption was only able to detect NPY in a very small subset of their participants, and the blood NPY levels were found to remain relatively constant throughout the 24-hour period, fluctuating 72 pg/ml (Sehested et al., 1992). No known studies describe the changes in blood NPY levels in response to food deprivation in humans.

The second hormone is liver-expressed antimicrobial peptide 2 (LEAP2), which was first described in the literature in 2003, and is a 3 to 4.5 kD peptide that was found in human blood, with DNA originally isolated from the liver (Krause et al., 2003). Further study found LEAP2 RNA expressed in human tissue from the small intestine, liver, kidney, and bladder (Howard et al., 2010). A later study showed the highest expression of mouse LEAP2 RNA in the jejunum of the small intestine, followed by the duodenum, liver, and then the ileum (Ge et al., 2018). Little information has been reported regarding the physiological functioning of LEAP2 beyond its antimicrobial properties, but further study of LEAP2 has found that it is an antagonist of the ghrelin receptor (Ge et al., 2018).

Based upon the discovery of this relationship between LEAP2 and ghrelin, two studies have found that LEAP2 levels in the blood decrease after a 24-hour fast in mice, and in opposition to the change in blood ghrelin levels (Ge et al., 2018; Mani et al., 2019). Additionally, Ge et al showed that blood LEAP2 levels increase after refeeding (Ge et al., 2018). And interestingly, Mani et al found that LEAP2 prevents ghrelin from activating NPY neurons in the arcuate nucleus of the brain (Mani et al., 2019). Furthermore, Mani et al showed a relationship between blood LEAP2 levels and blood glucose levels where mice given glucose orally had higher blood levels of LEAP2, and in fasted, obese humans

lower blood glucose levels were moderately correlated with lower blood LEAP2 levels (Mani et al., 2019). No known studies describe the changes in blood LEAP2 levels in response to food deprivation in humans. Additionally, no information is currently available regarding the 24-hour circadian pattern of LEAP2 levels in the blood.

This information seems to suggest that NPY and LEAP2 are two important hormones involved in the physiology of food deprivation. In animals they respond in opposite directions during food deprivation and, similar to the other hormones noted previously, they both appear to be influenced by blood glucose levels.

In summary, it appears that there are a variety of physiological responses to food consumption and food deprivation. It would seem that the primary stimulus driving these physiological responses is post-prandial nutrient absorption, namely changes in blood glucose. These physiological responses demonstrate that the body is: 1) very sensitive to changes in blood glucose levels, and 2) focused on maintaining blood glucose levels within a tight range.

2.3 Food Deprivation's Effect on Cardiovascular Physiology

As mentioned previously, there are currently around 17 published research articles that focus on the basic science of food deprivation and its effect on cardiovascular physiology in healthy human participants. The majority of these articles (13 of 17) studied acute food deprivation, and the remaining articles studied chronic food deprivation.

The first published article concerning food deprivation and cardiovascular physiology was published in 1954 (Jungmann, 1954), only in German, and then there was a 56 year gap in published studies until the 2000's. The remaining 16 articles were published from 2000 to 2018 (Cansel et al., 2014; Chan et al., 2007; Heilbronn et al., 2005; Horne et al., 2013; Mitchelmore, Stoner, Lambrick, Jobson, & Faulkner, 2018; Muthusami et al., 2017; Ohara et al., 2015; Samad et al., 2015; Schulz et al., 2015; Seker et al., 2017; Solianik et al., 2016; Stoner et al., 2017; Su et al., 2000; Tanaka, Tomoto, & Sugawara, 2016; Tsukamoto, Hitosugi, & Yokoyama, 2017; Y. Young et al., 2015).

The research studies described in these articles employed a variety of techniques to assess the effect of fasting on cardiovascular physiology in a variety of areas, and they produced the following results.

Fourteen of the aforementioned articles present results describing the impact of fasting on basic hemodynamics.

In regard to acute food deprivation, Muthusami et al studied children and young adults who fasted for at least 6 hours and then consumed a meal, and the study found that cardiac output was slightly lower pre-prandially (Muthusami et al., 2017). Tsukamoto et al evaluated patients who had undergone preoperative fasting for, on average, 3.43 hours (fluids) and 12.64 hours (solids); this study found that if vasopressor drugs were used to counteract anesthesia induced hypotension then they were used in the patients that fasted the longest, and that cardiovascular parameters were stated to remain stable throughout the surgery for all patients (Tsukamoto et al., 2017). Stoner et al performed an evaluation

of pulse waveform separation analysis done on individuals who had fasted for 12 hours and found that fasting led to a slightly decreased heart rate (Stoner et al., 2017). Mitchelmore et al conducted oscillometric pulse wave analysis in individuals over 50 years of age who had fasted for 12 hours and found that systolic blood pressure and heart rate were slightly decreased after fasting, whereas diastolic blood pressure was slightly increased after fasting (Mitchelmore, Stoner, Lambrick, Jobson, et al., 2018). Ohara et al studied the effect of eating (relative to continuing to fast) following a 12-hour fast on cardiovascular response in females and how that might be further influenced by menstrual cycle phase; this study found that after consuming a meal from a fasted state: 1) heart rate and systolic blood pressure increased in both the follicular and luteal phases, and 2) diastolic blood pressure decreased in both the follicular and luteal phases (Ohara et al., 2015). Schulz et al found that 18 hours of food deprivation in females led to increased heart rate during the midfollicular phase of the menstrual cycle, and decreased heart rate during the midluteal phase of the menstrual cycle (Schulz et al., 2015). Horne et al found that a 24-hour fast led to a decrease in systolic blood pressure, and an increase in diastolic blood pressure (Horne et al., 2013). Solianik et al evaluated the effect of a 48-hour fast in male amateur weight lifters, and found that fasting led to decreased blood pressure and heart rate (Solianik et al., 2016). Chan et al focused on the effect of a 72-hour fast, and the primary findings of this study were that a 72-hour fast led to increased heart rate and blood pressure (Chan et al., 2007). Lastly, Tanaka et al performed a case report of the effects of one male completing a one-week Buddhist fasting ritual (Danjiki); this study found that the one week fast led to: 1) decreased heart rate, and left ventricular

ejection time, and 2) increased left ventricular pre-ejection period, and systolic blood pressure variability power (Tanaka et al., 2016).

In regard to chronic food deprivation, Heilbronn et al evaluated a group of people who completed 22 days of an alternate day, 24-hour fasting program, and this study found that this ~3-week fasting program led to no changes in baseline blood pressure (Heilbronn et al., 2005). Cansel et al showed decreased heart rate during Ramadan, compared to after Ramadan (Cansel et al., 2014). Samad et al focused on evaluating blood pressure in males, before and throughout Ramadan, and found: 1) throughout Ramadan, end of day fasted state blood pressure and heart rate values were lower than what they would be two hours later after eating, and 2) systolic blood pressure increased the first half of Ramadan, and then decreased back to initial values the second half of Ramadan (Samad et al., 2015). Lastly, Seker et al measured 24-hour blood pressure in normotensive individuals who were and who were not participating in Ramadan, and the study found that systolic blood pressure was higher in individuals who were participating in Ramadan, and for those that were participating, systolic blood pressure was also elevated at the end of the daylight fasting period (Seker et al., 2017).

Five of the articles focused on the effect of food deprivation on heart rate variability (HRV). Two studies found an overall decrease in HRV, after a 72-hour fast (Chan et al., 2007), and after a one-week fast (Tanaka et al., 2016). Additionally, two different studies found an overall increase in HRV, after a 48-hour fast (Solianik et al., 2016), and during Ramadan (Cansel et al., 2014). Lastly, one study found a decrease in the high frequency

component of HRV after consuming a meal following a 12-hour fast, when compared to continuing to fast (Ohara et al., 2015).

Three of the articles focused on arterial stiffness. Two articles performed pulse wave analysis and showed that a 12-hour fast led to a decrease in arterial stiffness (Stoner et al., 2017; Y. Young et al., 2015). The third article showed an increase in arterial stiffness after a one-week fast (Tanaka et al., 2016).

Two articles focused on blood flow. In one article, Su et assessed the pulse spectrum harmonics in individuals that fasted for 24 hours, and this study found that the pulse spectrum harmonics indicated no difference in load on the heart when comparing fasted and fed states, but that the fasted state led to a shift in the qi away from the spleen and stomach and toward the kidney, thus indicating a change in physiological attention during fasting (Su et al., 2000). The second article found that consuming a meal after fasting for at least 6 hours led to increased abdominal blood flow volume with no change in cerebral blood flow volume (Muthusami et al., 2017).

Finally, one article focused on electrical activity of the heart and brain. In this article, Schulz et al evaluated the relationship between electrocardiography and electroencephalography after 18 hours of food deprivation in females, and found that 18 hours of food deprivation led to increased heartbeat evoked potentials activity (Schulz et al., 2015).

These research studies showed that the physiological effect of food deprivation on the cardiovascular system is complex, in part, because there is a great deal of variety in

specific fasting protocols. The majority (13 out of 17) of the aforementioned studies utilized an acute fast, with the remaining four utilizing a chronic fasting program (ex. Ramadan, alternate day fasting). Additionally, the majority (12 out of 17) of the studies employed a fasting duration of 24 hours or less, ranging from as few as 6 hours up to 24 hours. The remaining five employed a fasting duration of 2 to 7 days. Regardless, it is important to note that such variety in fasting duration complicates the understanding of how, specifically, food deprivation affects cardiovascular physiology. For example, one review paper highlights the almost constantly changing metabolic physiology as fasting duration progresses (Stockman et al., 2018).

The main takeaways from these studies are as follows. One, when comparing pre and post fasting it would appear that food deprivation generally has an excitatory effect on cardiovascular variables such as heart rate and blood pressure. A second takeaway is that it seems that being in a fasted state and then consuming food also has an excitatory effect on cardiovascular variables. Thirdly, it appears that fasting leads to a shift in blood flow away from the digestive tract. And lastly (fourthly), it seems that fasting generally leads to a decrease in arterial stiffness.

The tables below show the key results of the collection of previously published articles concerning the impact of acute (table 2.1) and chronic (table 2.2) food deprivation on cardiovascular physiology in healthy humans.

Table 2.1: Summary of previously published research regarding the cardiovascular response to acute food deprivation in healthy humans

A Summary of Previous Research on the Effect of Acute Fasting in Healthy Humans					
Fasting Duration	Systolic BP	Diastolic BP	Heart Rate	HR Variability	Source
12 hours	↓ (supine) ↓ (seated)	↔ (supine) ↑ (seated)	↓ (supine) ↓ (seated)	- -	Young, 2015
	↓ (supine) ↑ (seated)		↓ (supine) ↓ (seated)	- -	Stoner, 2017
18 hours	-	-	↑ (mid-follicular)	-	Schulz, 2015
	-	-	↓ (mid-luteal)	-	
24 hours	-	-	↑	↓	Herbert, 2012
	↓	↑	-	-	Horne, 2013
48 hours	↓	↓	↓	↑	Solianik, 2016
72 hours	↑	↑	↑	↓	Chan, 2007
1 week	↑	↑	↓	↑	Tanaka, 2016

Table 2.2: Summary of previously published research regarding the cardiovascular response to chronic food deprivation in healthy humans

A Summary of Previous Research on the Effect of Chronic Fasting in Healthy Humans					
Protocol	Systolic BP	Diastolic BP	Heart Rate	HR Variability	Source
Ramadan	-	-	↓	↑	Cansel, 2014
	↓	↓	-	-	Samad, 2015
	↑	↑	-	-	Seker, 2017
Alternate Day Fasting	↔	↔	-	-	Heilbronn, 2005

2.4 Proposed Mechanisms of Food Deprivation Altering Cardiovascular Physiology

Why might these changes to cardiovascular physiology be happening because of food deprivation?

When considering all of the current evidence, it is possible that the excitatory effect of acute food deprivation on cardiovascular physiology is linked to an increase in sympathetic activity. Due to limited evidence, it is difficult to say whether or not food deprivation is constantly sympatho-excitatory, when this sympatho-excitation specifically occurs, and for how long this sympatho-excitatory state(s) lasts. What the current research does show is that there is an excitatory effect on cardiovascular physiology post-prandially that lasts at least 1-3 hours (Ohara et al., 2015; Samad et al., 2015; Seker et al., 2017), and there is an excitatory effect of food deprivation that is revealed after a fast as short as 18 hours (Schulz et al., 2015) and after a fast as long as 72 hours (Chan et al., 2007). Previous research has also clearly established a link between increased sympathetic activity and increased cardiovascular activity (Miki & Yoshimoto, 2013).

What then connects the link from food deprivation to increased sympathetic activity to increased cardiovascular activity?

The primary initial effect of food consumption cessation appears to be the end of food intake that leads to a change in the volume of physical food in the digestive system, and a change in nutrient availability. As stated previously, a healthy adult human who has

regular access to food and consumes food regularly, will typically wait 4 to 7 hours between eating meals (Ishihara et al., 1985). Additionally, this is in agreement with the ~7 hours it takes a meal to travel through the stomach and small intestine after it has been consumed (Fischer & Fadda, 2016; Lawrence et al., 1996; Sugita et al., 2019). Therefore, this 7-hour post-prandial period is when the body experiences the receival and exit of food associated with nutrient absorption, and then anticipates the next bout of food consumption. One could then surmise that following this initial 7-hour post-prandial period, with no return to food consumption, food deprivation would begin to become an abnormal stressor.

What then is happening, physiologically, during and after this 7-hour post-prandial period, and how may that be linked to changes in cardiovascular physiology?

My speculation for how food deprivation affects cardiovascular physiology is based on molecular mechanisms. I speculate that the food deprivation-elicited-decrease in blood glucose leads to an increase in blood ghrelin concentration, a decrease in blood LEAP2 concentration, and an increase in blood NPY concentration. Furthermore, I speculate that these changes in blood hormone concentrations will be associated with an excitatory effect on cardiovascular activity, linked through downstream molecular mechanisms.

As stated previously, food deprivation leads to a brief initial rise in blood glucose, followed by a decrease and then stable maintenance that, for the most part, evades hypoglycemia (Hojlund et al., 2001). Additionally, food deprivation leads to an increase in ghrelin (Shiiya et al., 2002); specifically, ghrelin seems to be responsive to changes in

blood glucose (Broglio et al., 2004). Furthermore, LEAP2 functions as an endogenous antagonist of the ghrelin receptor (Ge et al., 2018), and has been shown to decrease with food deprivation, in animals (Mani et al., 2019). A decrease in LEAP2 may mean that food deprivation allows for the body to have a more robust response to ghrelin, even if ghrelin levels remain unchanged. Food deprivation has also been shown to lead to an increase in NPY, in animals (Yoshihara et al., 1996), which is likely due to decreased NPY inhibition because of decreased blood glucose (Burdakov et al., 2005).

There are two possible pathways that link changes in blood hormone concentrations to changes in cardiovascular activity: the ghrelin pathway, and the NPY pathway.

For the ghrelin pathway, I speculate that the increased concentration of blood ghrelin and/or the increased responsiveness to ghrelin precipitates an increase in corticotropic releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), which facilitates an increase in cortisol that leads to both gluconeogenesis (to counteract the decrease in blood glucose) and changes in nitric oxide synthase (NOS) levels by decreasing neuronal NOS (nNOS) and increasing inducible NOS (iNOS), that then lead to increased sympathetic nervous system activity, which has an excitatory effect on cardiovascular activity (ex. increased blood pressure and heart rate). The current evidence for this pathway is as follows.

Research has shown that ghrelin can stimulate both CRH (Cabral, Portiansky, Sanchez-Jaramillo, Zigman, & Perello, 2016) and ACTH (Milosevic et al., 2013), and both CRH and ACTH have also been shown to increase due to food deprivation (Djordjević et al.,

2008; Yadawa & Chaturvedi, 2016). Additionally, CRH has been shown to directly stimulate ACTH (Pecori Giraldi et al., 2011), and then ACTH subsequently directly stimulates cortisol (Novoselova et al., 2019). Cortisol, too, has been shown to increase with food deprivation (Bergendahl, Vance, Iranmanesh, Thorner, & Veldhuis, 1996; Espelund et al., 2005). And interestingly, there is evidence that cortisol and ghrelin have both an oppositional relationship (Espelund et al., 2005) and a positive correlation, with cortisol having a direct effect on ghrelin (Azzam, Gilad, Limor, Stern, & Greenman, 2017). Besides having an effect on ghrelin, cortisol has also been shown to directly facilitate the decrease in brain nNOS that comes with food deprivation (Kim, Lee, Choi, Kim, & Jahng, 2004). Furthermore, food deprivation has been shown to decrease nNOS in the brain (Ueta, Levy, Chowdrey, & Lightman, 1995), and decrease nNOS and increase iNOS in the jejunum (Ito, Uchida, Yokote, Ohtake, & Kobayashi, 2010). Additionally, nNOS and iNOS are understood to have an oppositional relationship with sympathetic nervous system activity where increased sympathetic activity is associated with decreased nNOS (Y. Wang & Golledge, 2013) and increased iNOS (Smith et al., 2011).

For the NPY pathway, I speculate that the increase in blood NPY concentration precipitates an increase in epinephrine, along with NPY's known direct excitatory effect on cardiovascular activity. The increase in epinephrine would facilitate gluconeogenesis (to counteract the decrease in blood glucose) and lead to an excitatory effect on cardiovascular activity. The current evidence for this pathway is as follows.

Research has linked NPY to adrenal epinephrine production and blood glucose maintenance, in animals (M. Wang et al., 2016). Additionally, NPY has been shown to cause vasoconstriction (Hanko et al., 1986), in addition to increasing blood pressure with infusion (Allen & Bloom, 1985).

Lastly, it should also be noted that these ghrelin and NPY pathways likely do not act independently. Research has shown that ghrelin can directly stimulate NPY (Hashiguchi et al., 2017), and that NPY can directly stimulate CRH and ACTH (Haas & George, 1989). Furthermore, it is likely that other factors beyond blood glucose are influencing ghrelin and NPY. As an example, there is evidence that both ghrelin and NPY are decreased/inhibited by insulin (Belgardt et al., 2009; Broglio et al., 2004) and insulin has been shown to decrease during food deprivation, in tandem with the decrease in blood glucose (Broglio et al., 2004; Hojlund et al., 2001).

The evidence presented appears to support the notion that ghrelin and NPY are significant upstream factors that respond directly to food deprivation, and then could lead to a chain of downstream effects that modulate cardiovascular physiology.

2.5 Summary

In summary, food deprivation research has been occurring for over 300 years and much has been learned, but there still remains a large gap in knowledge regarding the impact of food deprivation on cardiovascular physiology. Generally, when the consumption of food ceases, it is the loss of food and the loss of nutrient availability, primarily glucose,

that drives the physiological effects of food deprivation. When considering cardiovascular physiology, the effects of food deprivation appear to be sympatho-excitatory. And why food deprivation causes sympatho-excitation of the cardiovascular system is likely due to modulation of molecular factors stemming from the body's focus on managing blood glucose levels.

3 Methodology

The previous chapter focused on a detailed recounting of the current state of knowledge in the field of food deprivation research, specifically in regard to the impact of food deprivation on cardiovascular physiology. This chapter serves to describe the methodology that was utilized to investigate some existing limitations in knowledge regarding the impact of food deprivation on cardiovascular physiology.

3.1 Hypothesis

The two-part hypothesis of this dissertation was:

1) It was hypothesized that a 24-hour bout of acute food deprivation would likely lead to an excitatory cardiovascular response in healthy humans, associated with hormonal changes. It was expected that blood pressure and heart rate would be increased after 24 hours of acute food deprivation, along with decreased blood glucose, increased plasma ghrelin, increased plasma NPY, and decreased plasma LEAP2.

2) It was further hypothesized that experiencing this 24-hour bout of acute food deprivation twice per week for 4 weeks would lead to cardiovascular adaptations stemming from reduced basal sympathetic tone and altered circulating hormone levels. It was expected that blood pressure and heart rate would be decreased after 4 weeks of chronic food deprivation, along with increased heart rate variability, decreased plasma ghrelin, decreased plasma NPY, and increased plasma LEAP2.

3.2 Specific Aims

This dissertation focused on two specific aims.

Specific aim one was to evaluate the hemodynamic and molecular changes, related to cardiovascular physiology, that occurred within and following a 24-hour acute period of food deprivation. The primary outcome variables for specific aim one were: blood pressure, heart rate, and blood biomarkers.

Specific aim two was to evaluate the autonomic, hemodynamic, and molecular changes, related to cardiovascular physiology, that occurred during and following a 4-week period of a chronic food deprivation regimen. The primary outcome variables for specific aim two were: blood pressure, heart rate (to include heart rate variability), and blood biomarkers.

3.3 Experimental Protocol

The experimental protocol for this dissertation research project is detailed below in two sections. The first section articulates the details of the original protocol design, and the second section articulates the details of the COVID-19 pandemic contingency plan design.

3.3.1 Original Protocol Design

The original protocol design for this research project employed a repeated-measures longitudinal crossover design where participants underwent a 4-week control period

followed by a 6-week treatment period (10 weeks total). Both the control and treatment periods began with a 24-hour acute phase, followed by the remaining time being a chronic phase (figure 3.1).

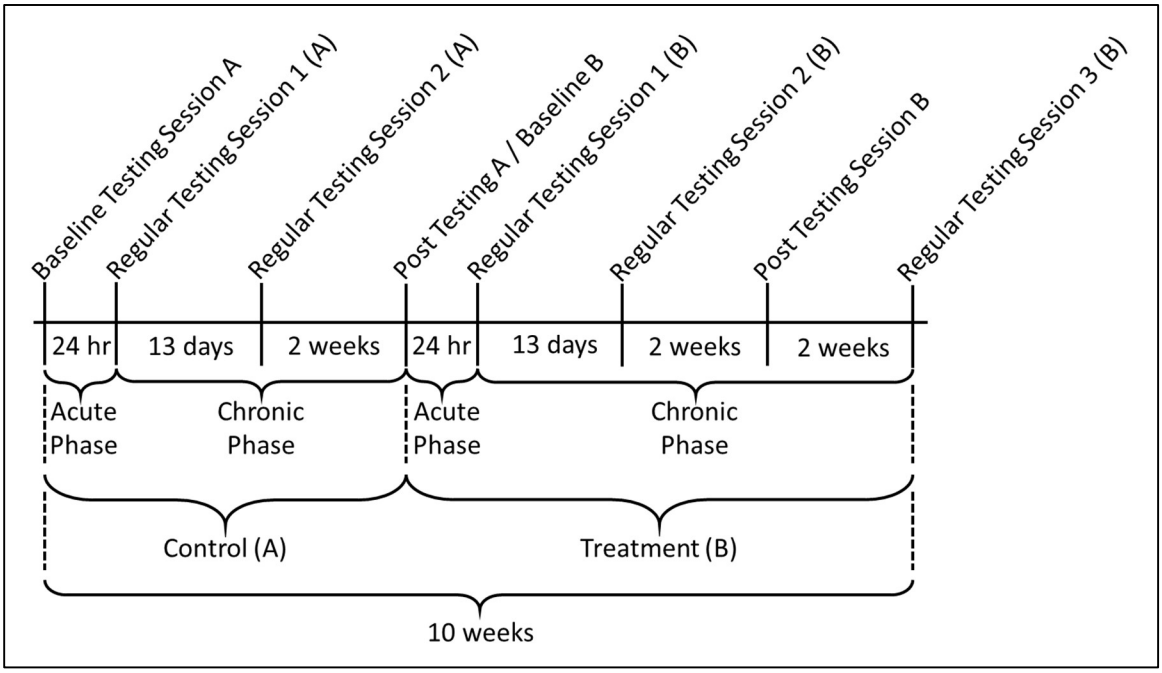


Figure 3.1: Experimental timeline (original protocol)

Participants were asked to come to the lab a total of 8 times over the course of 10 weeks. Their first visit was a baseline testing session for the control condition, followed by a regular testing session after 24 hours, and then another regular testing session 2 weeks after the baseline testing session. Four weeks after the baseline testing session participants were asked to return to the lab for a post testing session for the control condition which also served as the baseline testing session for the treatment condition. The treatment condition testing sessions mimicked the control condition for the first 4

weeks, with the addition of one regular testing session 2 weeks after the post testing session for the treatment condition.

Each baseline and post testing session included measurements of anthropometric and hemodynamic characteristics followed by a finger poke (one drop of blood) and venous blood draw (18 ml of blood), and then an autonomic function test before finishing with an assessment of body composition prior to a meal and participant departure. The regular testing sessions included everything that was included in the baseline/post testing sessions except for the autonomic function test, assessment of body composition, and meal.

The anthropometric characteristics measured were height and mass. The hemodynamic characteristics measured were heart rate (HR) and blood pressure (BP). The finger poke was utilized to assess blood glucose levels, and the venous blood draw was utilized to acquire blood samples for ELISAs. The autonomic function test included electrocardiography (EKG), beat-to-beat blood pressure (BTBBP), respiration rate (RR), and muscle sympathetic nerve activity (MSNA) measurements. Body composition was assessed via a full body dual energy x-ray absorptiometry (DEXA) scan.

A figure (figure 3.2) comparing the components of the different testing sessions is shown below, followed by a detailed description of the testing sessions.

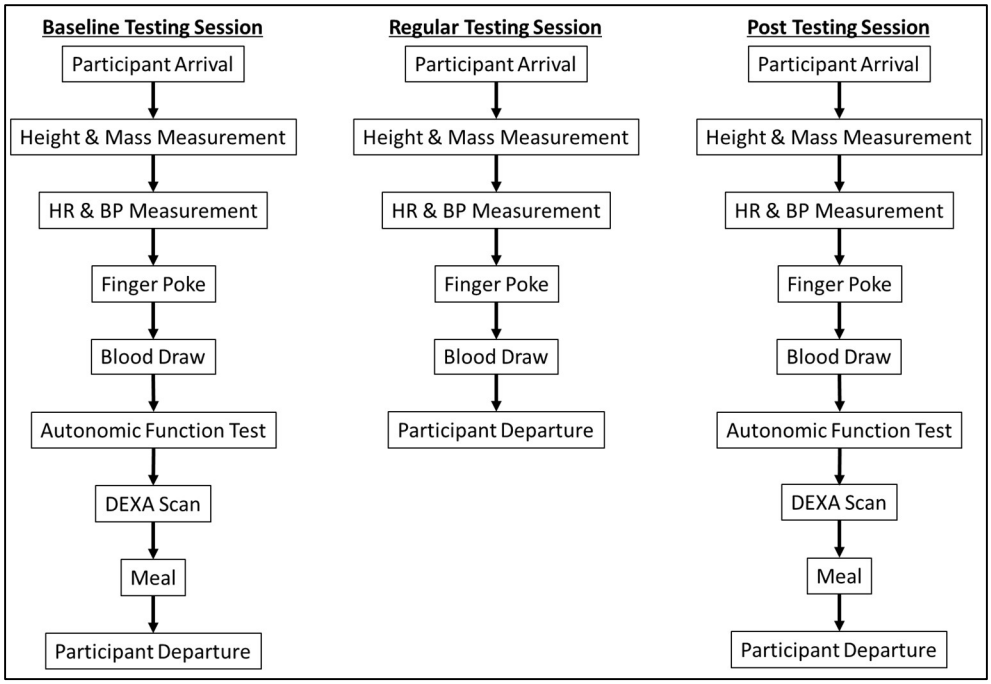


Figure 3.2: Testing session components (original protocol)

When arriving at the lab for testing sessions, participants were asked to arrive in the morning, well hydrated, in a fasted state, and around their usual breakfast time. Participants were also asked to wear comfortable attire, including a short-sleeved shirt and shorts, and to consume no stimulants (ex. caffeine) after waking and prior to the testing session.

Upon arrival to the lab for a baseline or post testing session participants were directed to remove their shoes and empty their pockets before having their height and mass measured. Then they were asked to sit quietly for 5 minutes before resting BP and HR were measured in a seated position. Next was a finger poke and a venous blood draw. Participants were then instrumented for EKG, BTBBP, RR, and MSNA for the autonomic function test. The autonomic function test lasted for 10 minutes while

participants breathing rate was guided with a metronome at a frequency of 15 breaths per minute. Participants were then de-instrumented, escorted down the hall for the DEXA scan, and then returned to the lab where they were given a standardized meal.

The baseline/post testing session concluded with the meal, and then participants were thanked and reminded of their next scheduled appointment (if applicable) before they departed.

In addition to the above, the baseline testing sessions (control and treatment) also ended with participants given ambulatory blood pressure monitors to wear for 24 hours, until they arrived for their post-baseline regular testing session. Participants were asked to refrain from exercise and stimulants during these 24-hour periods.

The protocol for a regular testing session mimicked the protocol for a baseline/post testing session, except that a regular testing session did not include an autonomic function test, assessment of body composition, or a meal.

The baseline and post testing sessions lasted ~1.5 hours, and the regular testing sessions lasted ~30 minutes. The total time commitment over the entire 8-visit 10-week testing period was ~7 hours.

During the entire 10-week testing period participants were asked, to the best of their ability, to maintain their current lifestyle with no significant changes to their diet, activity level, or stress level, and no experimentally controlled changes were instituted during the 4-week control condition. During the treatment condition, participants were asked to

participate in a 24-hour period of food deprivation (water intake only) for the acute phase (between baseline testing and regular testing session 1), and then they were also asked to participate in a period of 24-hour food deprivation (water intake only) twice per week (on non-consecutive days) for the entire 6-week treatment condition. Each period of 24-hour food deprivation occurred as a breakfast to breakfast fast where participants were asked to eat breakfast, and then withhold eating until breakfast the following day. During the treatment condition participants were also asked to have two 24-hour periods of normal eating between the 24-hour periods of food deprivation (ex. fasting on Tuesdays and Fridays). In the lab, participants were provided a standardized breakfast of oatmeal after their pre-acute phase baseline testing session during both the control and treatment conditions. Additionally, throughout the entire 10-week experimental timeline participants were surveyed about their dietary intake (acute phases only), activity level (every two weeks), and stress level (every two weeks). Demographic characteristics were also collected on participants.

3.3.2 COVID-19 Pandemic Contingency Plan

Due to state-wide and university-level restrictions in response to the COVID-19 (SARS-CoV-2) global pandemic, data collection for the original experimental protocol was halted and not able to be completed. Therefore, the following contingency plan was developed to be utilized to accompany any data from the original experimental protocol. The pandemic contingency plan was designed with four components.

3.3.2.1 Pandemic Contingency Plan Component One

Pandemic contingency plan component one was an exact replication of the full 10-week original experimental protocol, but with just two participants (one female, one male), to constitute a case series. The change in the number of participants allowed to participate was in accordance with stipulations imposed by the Institutional Review Board.

3.3.2.2 Pandemic Contingency Plan Component Two

Pandemic contingency plan component two was solely focused on the acute phases of the original experimental protocol. This component employed a repeated-measures crossover design where participants underwent a 24-hour control acute phase, followed by a period of reprieve, and then a 24-hour treatment acute phase. The period of reprieve lasted anywhere from a few days up to a few weeks, depending on participant availability, with both acute phases occurring within 4 weeks of each other (figure 3.3).

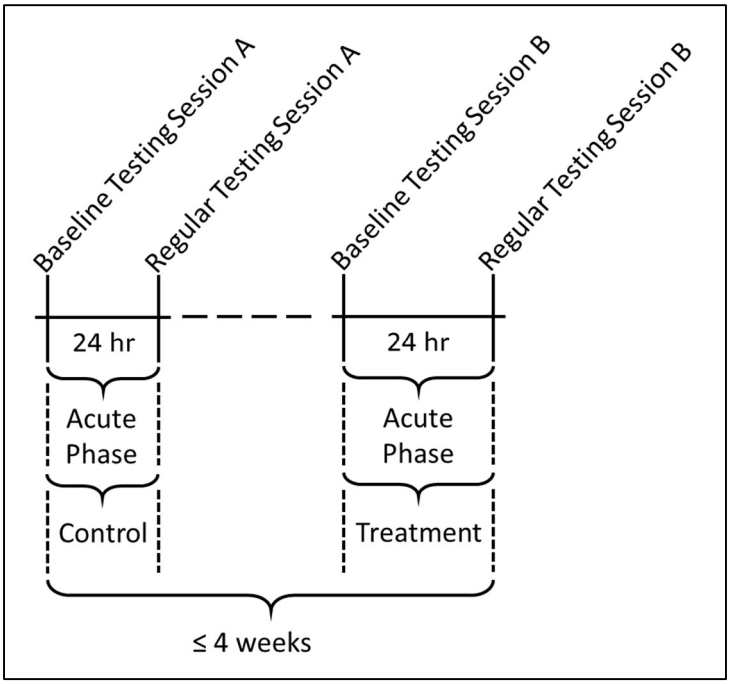


Figure 3.3: Experimental timeline (contingency plan component two)

For this component, participants were asked to visit the lab a total of 4 times. The first visit was a baseline testing session for the control condition (A), followed by a regular testing session after 24 hours. The third visit was a baseline testing session for the treatment condition (B), followed by a regular testing session after 24 hours. Baseline testing session A lasted ~45 minutes, and the remaining visits lasted ~30 minutes each. The total time commitment for all 4 visits was 2.25 hours.

In terms of testing session components, all four lab visits/testing sessions mimicked the regular testing sessions in the original experimental protocol, with some additions to the two baseline testing sessions. Baseline testing session A included a DEXA scan and meal, and baseline testing session B included a meal. These components are outlined in the figure (figure 3.4) below.

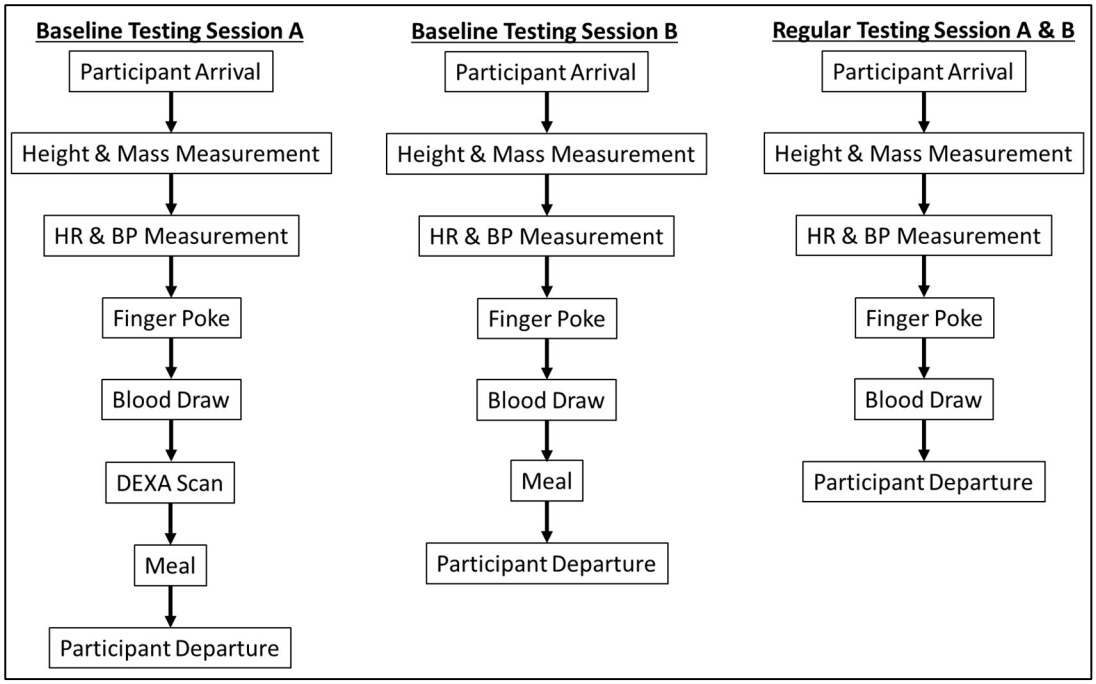


Figure 3.4: Testing session components (contingency plan component two)

Furthermore, the baseline testing sessions (control and treatment) also ended with participants given ambulatory blood pressure monitors to wear for 24 hours, until they arrived for their post-baseline regular testing session. Participants were asked to refrain from exercise and stimulants during these 24-hour periods. Additionally, demographic information was collected on participants, and participants were surveyed about their dietary intake (acute phases only), activity level, and stress level.

When arriving at the lab for testing sessions, participants were asked to arrive in the morning, well hydrated, in a fasted state, and around their usual breakfast time. Participants were also asked to wear comfortable attire, including a short-sleeved shirt, and to consume no stimulants (ex. caffeine) after waking and prior to the testing session.

During the control condition, participants were asked to maintain their normal eating habits, and during the treatment condition, participants were asked to participate in a 24-hour period of food deprivation (water intake only) from baseline testing session B to regular testing session B.

3.3.2.3 Pandemic Contingency Plan Component Three

Component three was solely focused on the chronic portion of the treatment phase of the original experimental protocol. This component employed a repeated-measures longitudinal design where participants underwent a 4-week experimental timeline (figure 3.5).

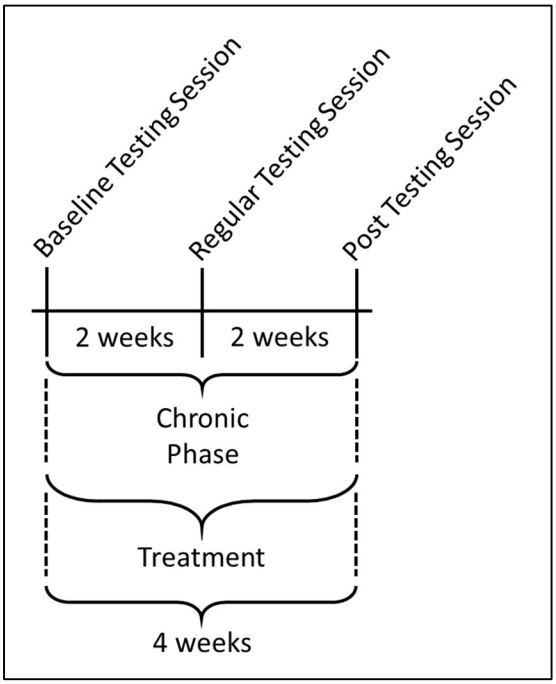


Figure 3.5: Experimental timeline (contingency plan component three)

For this component participants were asked to visit the lab a total of 3 times over the course of 4 weeks. The first visit was a baseline testing session, followed by a regular testing session 2 weeks later, and then a post testing session 2 weeks after that. The baseline testing session and the post testing session lasted ~1.5 hours, and the regular testing session lasted ~30 minutes. The total time commitment for all 3 visits was 3.5 hours.

In terms of testing session components, all testing sessions were exact replicas of their respective testing session from the original experimental protocol, except for the omission of the meal from the baseline and post testing sessions (figure 3.6).

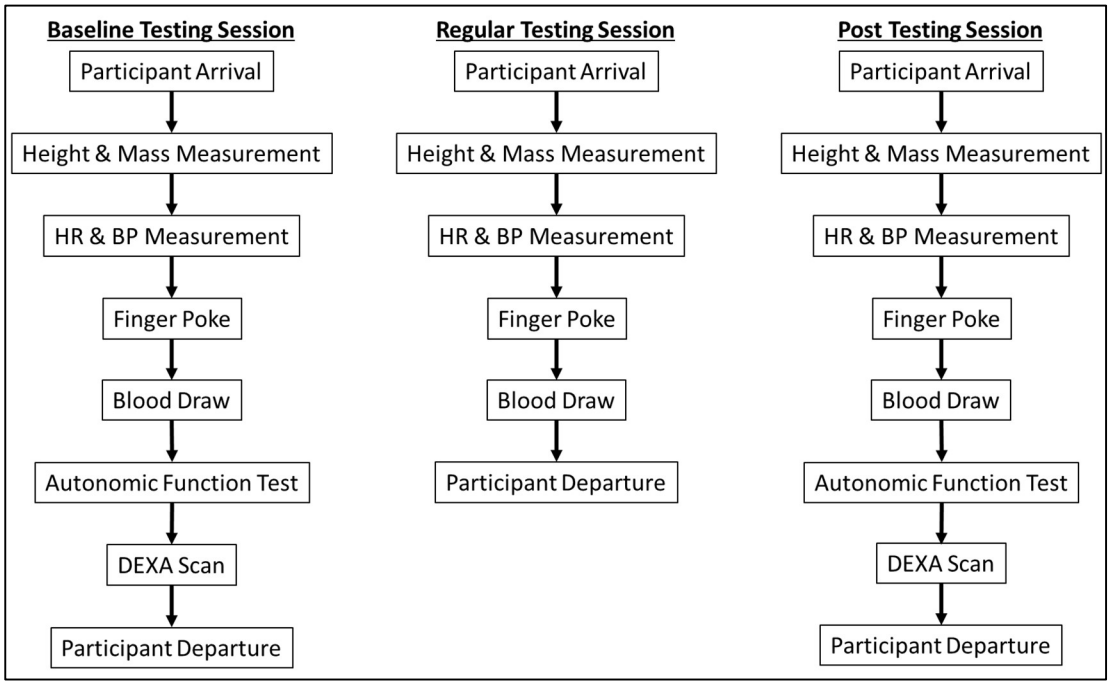


Figure 3.6: Testing session components (contingency plan component three)

When arriving at the lab for testing sessions, participants were asked to arrive in the morning, well hydrated, in a fasted state, and around their usual breakfast time.

Participants were also asked to wear comfortable attire, including a short-sleeved shirt, and to consume no stimulants (ex. caffeine) after waking and prior to the testing session.

During the entire 4-week treatment condition experimental timeline, participants were asked to participate in a 24-hour period of food deprivation (water intake only) twice per week, on non-consecutive days. Each period of 24-hour food deprivation occurred as a breakfast to breakfast fast where participants were asked to eat breakfast, and then withhold eating until breakfast the following day. Additionally, participants were also asked to have two 24-hour periods of normal eating between the 24-hour periods of food deprivation (ex. fasting on Tuesdays and Fridays). Furthermore, demographic characteristics were collected on the participants, and throughout the entire 4-week experimental timeline participants were surveyed at each visit about their activity level and stress level.

3.3.2.4 Pandemic Contingency Plan Component Four

Component four was performing an in-depth review of current food deprivation research to explore the existing knowledge on how food deprivation impacts cardiovascular physiology (specifically blood pressure and heart rate), both acutely and chronically.

To acquire articles for this in-depth review an exhaustive search of the PubMed online database was performed using a combination of both food deprivation and cardiovascular physiology article title search terms. The food deprivation search terms that were utilized were: food deprivation, fasting, intermittent fasting, and caloric restriction. The

cardiovascular physiology search terms that were utilized were: heart rate and blood pressure.

Once the initial PubMed was complete, filtering was applied to exclude review articles, systematic reviews, and meta-analyses, and only include articles written in English that focused on human adult participants.

The remaining results were then individually assessed to determine whether or not they met the criteria of being an original research article that evaluated how food deprivation (acute or chronic) effected cardiovascular physiology (heart rate and/or blood pressure) in adult humans.

Once the final list of articles was generated, the articles were divided into categories based upon whether or not they employed an acute or chronic food deprivation experimental design. Once that division occurred, the articles were then sub-divided by the specifics of the food deprivation protocol (fasting duration, fasting frequency, etc) and the variables of interest (blood pressure and heart rate parameters) were extracted and divided based upon whether or not they came from healthy or non-healthy participants (figure 3.7). For this dissertation, only data from healthy participants were analyzed.

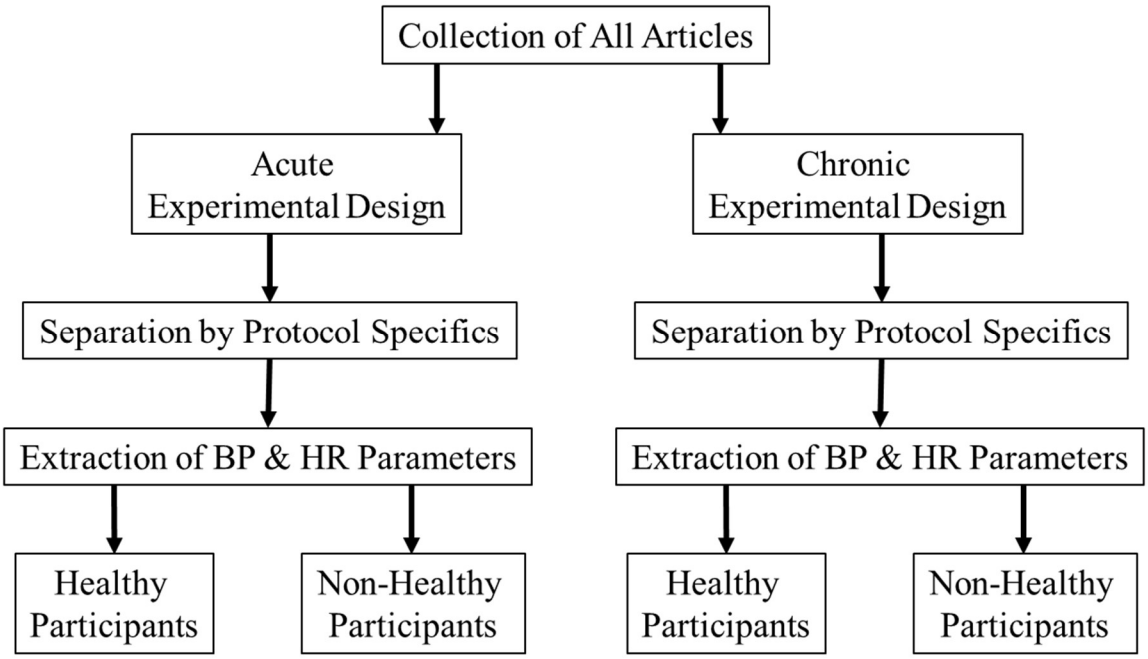


Figure 3.7: In-depth review article organization

3.4 Participants

3.4.1 Sample Size

The sample size goal of this research project for the original experimental protocol was to test a minimum of 14 participants, and a maximum of 20 participants, with an equal number of female and male participants.

The sample size goal for the pandemic contingency plan component one was 1 to 2 participants, with an equal number of female and male participants.

The sample size goal for the pandemic contingency plan component two was 14 to 20 participants, with an equal number of female and male participants.

The sample size goal for the pandemic contingency plan component three was 4 to 6 participants, with an equal number of female and male participants.

The sample size goal for the pandemic contingency plan component four was at least 2 articles with homogenous protocols and participant populations.

These sample size goals were derived through sample size analysis done via Microsoft Excel, SigmaPlot, and R. Estimated, needed sample size was calculated with data from previously published research, and set at an alpha level of 0.05 and a power of 0.7 to 0.9 . The variables utilized for the sample size analysis were blood pressure, heart rate, and blood ghrelin levels. These variables were utilized because already published data existed, and they represented primary outcome variables. The tables below show the results of the sample size analysis (table 3.1) as well as the previously published data that were utilized to calculate said sample sizes (tables 3.2 & 3.3).

Table 3.1: Results of sample size analysis utilizing previously published data

Results of Sample Size Analysis				
	Acute Protocol		Chronic Protocol	
	Power	Sample Size	Power	Sample Size
Systolic Blood Pressure	0.7	23	0.7	4
	0.8	30	0.8	4
	0.9	40	0.9	5
Heart Rate	0.7	10	0.7	-
	0.8	12	0.8	-
	0.9	16	0.9	-
Plasma Ghrelin	0.7	5	0.7	3
	0.8	6	0.8	4
	0.9	7	0.9	4

Table 3.3: Previously published data (acute food deprivation) that was utilized for sample size analysis

Previously Published Data for Acute Food Deprivation						
	Baseline		Fasted		Fasting Duration	Source
Blood Pressure	SBP (mmHg)		SBP (mmHg)		72 hours	Chan, 2007
	Average	104.5	Average	107.7		
	Sd	8.73	Sd	3.97		
Heart Rate	HR (bpm)		HR (bpm)		24 hours	Herbert, 2012
	Average	66.7	Average	71.3		
	Sd	5.27	Sd	6.38		
Plasma Ghrelin	Ghrelin (µg/l)		Ghrelin (µg/l)		24 hours	Espelund, 2005
	Average	0.31	Average	0.95		
	Sd	0.31	Sd	0.57		

Table 3.3: Previously published data (chronic food deprivation) that was utilized for sample size analysis

Previously Published Data for Chronic Food Deprivation						
	Before		After		Protocol	Source
Blood Pressure	SBP (mmHg)		SBP (mmHg)		1 mo of 25% caloric restriction 2 d/wk	Harvie, 2011
	Average	115.2	Average	111.6		
	Sd	2.00	Sd	1.80		
Plasma Ghrelin	Ghrelin (µg/l)		Ghrelin (µg/l)		1 mo of 25% caloric restriction 2 d/wk	Harvie, 2011
	Average	0.14	Average	0.16		
	Sd	0.01	Sd	0.01		

3.4.2 Recruitment Criteria

All female participants were recruited to begin the experimental protocol in the same phase of their menstrual cycle, and the following inclusion criteria were utilized to recruit participants (female and male).

- Aged 18 to 40 years
- Currently healthy (no known health conditions)
- No history of eating disorders (ex. anorexia, bulimia)
- Normal resting heart rate (50-100 bpm) and normotensive (< 130/80 mmHg)
- Not currently participating in a food deprivation regimen
- Not currently majorly injured or recovering from a major injury or surgery
- Do not smoke or vape nicotine products regularly (i.e. less than once a month)

- Pre-menopausal with a normal menstrual cycle (females only; cycle length 15-45 days)
- Not breast feeding, pregnant, or trying to become pregnant (females only)
- Not using hormone altering contraception (females only; ex. oral, injection, implant)

3.5 Data Collection Procedures

3.5.1 Demographics and Lifestyle Characteristics:

All participants self-reported their age, date of birth, biological sex, identity, and any known food allergies. The answers that the participants gave for their self-ascribed identity were organized into three categories: classification, personality, and appearance. Classification included answers such as race, ethnicity, sex, etc. Personality and appearance included answers that described either the participant's personality or appearance, respectively. Female participants were asked to self-report the first day of menstruation (menstrual cycle day one).

Throughout each acute phase, participants were asked to maintain a log of each time they ate with what they ate in item and quantity. At the conclusion of each acute phase, participants were asked to self-report at what time they went to sleep and at what time they woke up during the acute phase. Additionally, participants were asked to self-report the last time that they ate prior to each laboratory visit.

At the onset of participation, participants were asked to self-report the weekly duration of time they spend performing low intensity cardiorespiratory activities, moderate to vigorous intensity cardiorespiratory activities, and resistance activities. Additionally, every two weeks, during the chronic phases, participants were asked to self-report whether or not their physical activity level had changed as: no change at all, increased, or decreased. The physical activity duration answers were compared to the ACSM physical activity guidelines (Liguori & Medicine, 2020) in order to categorize participants into a particular fitness level (low, moderate, high). If a participant's physical activity met or exceeded all of the ACSM guidelines then they were classified in the high fitness level group, which was given a numeric value of '3'. If a participant's physical activity met some but not all of the ACSM guidelines then they were classified in the moderate fitness level group, which was given a numeric value of '2'. If a participant's physical activity did not meet any of the ACSM guidelines then they were classified in the low fitness level group, which was given a numeric value of '1'. Furthermore, the following numerical values were assigned when participants were asked if their physical activity level had increased (numeric value '1'), decreased (numeric value '-1'), or remained the same (numeric value '0').

Also at the onset of participation, participants were asked to self-report their current stress level in five areas. The five areas were: personal relationships, work, school, personal health, and relationship with society. For each area participants were asked to rank their current stress level as: not stressed at all, slightly stressed, moderately stressed, or severely stressed. Additionally, every two weeks, during the chronic phases,

participants were asked to self-report whether or not their overall stress level had changed as: no change at all, increased, or decreased. The stress level answers were converted from the qualitative answers to a 0 – 4 numeric scale with 0 representing "not stressed at all" to 4 representing "severely stressed". These numbers were used to quantify per area stress level and calculate total stress level. Furthermore, the following numerical values were assigned when participants were asked if their stress level had increased (numeric value '1'), decreased (numeric value '-1'), or remained the same (numeric value '0').

3.5.2 Anthropometrics

A physician's floor scale with a stadiometer was used to measure standing height and mass. A DEXA machine (GE Healthcare) was utilized to assess body composition at each baseline and post testing session, except for baseline testing session B within pandemic contingency plan component two. Each DEXA scan involved the participant lying supine for 6 minutes while their whole body was scanned. The DEXA scan provided whole-body body fat in percent, whole-body bone mineral content in grams, and whole-body bone mineral density in grams per centimeters squared.

3.5.3 Hemodynamics

At the beginning of each visit, after a 5-minute period of seated rest, an automatic blood pressure monitor (Omron) cuff was placed around the participant's upper arm, and blood pressure and heart rate were measured three times with one minute of rest between each measurement. An average was then calculated from those three measurements.

An ambulatory blood pressure monitor (ABPM) (Spacelabs Healthcare) cuff was fitted around the upper arm of the participant's non-dominant hand. The cuff was connected to and controlled by a small battery pack. The ABPM was set to measure the participants blood pressure and heart rate every 30 minutes throughout each 24-hour acute phase. The blood pressure and heart rate data were compiled and categorized by both protocol phase (control, treatment) and participant consciousness (awake, asleep). Data for a given category was only considered usable if at least 67% of the measurement time points had valid measurements. Furthermore, an outlier was considered to be any data point that was more than three standard deviations from the mean for a given category, and said outliers were identified and removed.

3.5.4 Blood Sampling

Once, at every visit, a small lancet (Linkfar) was used to puncture the skin at the lateral tip of the middle finger (third phalange) of the participant's non-dominant hand in order to procure one drop of blood onto the end of a blood glucose test strip (Accu-Chek Aviva) that was then inserted into a blood glucose monitor (Accu-Chek Aviva). The blood glucose monitor provided blood glucose concentration in milligrams per deciliter. Prior to the finger being punctured the site was cleaned with an alcohol wipe. Additionally, the first drop of blood procured after puncture was wiped away with gauze, and the second drop of blood was collected for analysis.

Also at every visit, a butterfly venipuncture needle (BD Vacutainer) was used to pierce an antecubital arm vein to collect ~20 ml of blood into two vacuum blood collection tubes (BD Vacutainer). The collected whole blood remained uncoagulated with EDTA

(18 mg) and lithium heparin (158 USP), one tube each. The uncoagulated blood was then centrifuged at 1500 rpm for 20 minutes at 4°C, and the non-hemolyzed plasma was aliquoted into micro-centrifuge tubes and stored frozen at -80°C until analyzed. If possible, up to 2 ml (1 ml per micro-centrifuge tube) of plasma were aliquoted from each whole blood collection tube.

The plasma samples (from EDTA blood collection tubes) were analyzed for concentrations of the following: ghrelin, LEAP2, and NPY. These targets were measured by following the provided instructions (appendix A) within the ELISA kits (Aviva Systems Biology). In brief, serially titrated standards (100 µl per well), blanks (100 µl per well), and plasma samples (100 µl per well, 25% dilution) were first added to a micro-well plate that was pre-coated with a capture antibody. Then a detector antibody was added to the micro-well plate, followed by a detection conjugate, and then a detection substrate. Typically, the micro-well plate underwent incubation after each addition, and was washed before each new addition. Appendix A of this dissertation provides the specific, detailed instructions of each assay procedure. After the final incubation, a stop solution was added to each well and the micro-well plate was read twice by a microplate reader (BioTek) for optical density at a wavelength of 450 nm. The initial optical density value for each well was calculated as the average of the two plate reading values, and relative optical density was then calculated as the optical density of a given well minus the average optical density of the blank wells. Afterwards, a standard curve was generated for the serially titrated standards, and linear regression was applied to calculate the concentration values in each plasma sample. All serially

titrated standards and blanks were made in quadruplicate, and all samples were made in duplicate.

3.5.5 Autonomic Function Testing

During each baseline and post testing session within the original experimental protocol and pandemic contingency plan components one and three, an autonomic function test period occurred that included the following instrumentation during a controlled breathing (15 breaths per minute) period at a state of rest.

Three EKG leads were placed on the anterior upper torso of the participant; one on each shoulder at the acromion, and one at the inferior lateral aspect of the left rib cage. A lead-2 EKG signal was derived from these EKG leads (Finapres Nova) and was transmitted into and displayed by multi-channel data acquisition and recording software (WinDaq). This EKG signal allowed for continuous measurement of the electrical activity of the heart in volts. Average heart rate and heart rate variability were derived from a 5-minute segment of the continuous EKG electrical activity data. Heart rate variability was derived in the time domain as R-to-R interval, and in the frequency domain for R-to-R interval as low frequency (0.04 Hz to 0.15 Hz), high frequency (0.15 Hz to 0.4 Hz), and low frequency to high frequency ratio. A Fourier transform was utilized to calculate the frequency data. The averages and calculations were derived using the WinCPRS software program.

Beat-to-beat blood pressure was recorded by a finometer (Finapres Nova) via a finger cuff placed around the middle phalanx of the middle finger (third phalange), of the right

hand. The BTBBP signal was transmitted into and displayed by multi-channel data acquisition and recording software (WinDaq). The finometer allowed for continuous measurement of arterial blood pressure in millimeters of mercury. Average systolic, diastolic, and mean arterial pressure were calculated from a 5-minute segment of the continuous data. These averages were derived using the WinCPRS software program.

A pneumobelt (Harvard) was placed around the participants torso at the level of the inferior aspect of the rib cage just superior to the EKG electrode/lead. The pneumobelt responded to changes in the diameter of the rib cage as the participant breathed. A signal to derive respiration rate was derived from these responses to changes in rib cage diameter and was transmitted into and displayed by multi-channel data acquisition and recording software (WinDaq). The pneumobelt allowed for continuous measurement of pneumobelt pressure in volts.

A tungsten recording microelectrode (FHC) was inserted into the skin at the area of the lateral, distal upper leg, posterior to the lateral epicondyle of the femur. After insertion, the microelectrode was manipulated until it came into contact with the peroneal nerve. Once it contacted the peroneal nerve the recording microelectrode received the nerve signal. The nerve signal was amplified, band-pass filtered, and integrated (Iowa Biosystems; Astro-Med) before being transmitted into and displayed by multi-channel data acquisition and recording software (WinDaq). This setup allowed for continuous measurement of nerve activity in volts. The continuous nerve data was used to identify bursts, or MSNA. These integrated bursts of MSNA were first identified by a computer and based on an approximate signal-to-noise ratio of 3:1 with a burst peak latency from

the proceeding R-wave of 1.3 seconds. The identification of the MSNA bursts allowed for the calculation of burst frequency in bursts per minute and burst incidence in bursts per 100 heart beats, from a 5-minute segment of the continuous data. All of this was derived using the WinCPRS software program.

Lastly, baroreflex sensitivity (BRS) was assessed in the time domain by calculating linear regressions among changes of systolic pressures and accompanying changes of R-R intervals (the sequence method) to calculate BRS for both up-up and down-down sequences. All of this was derived using the WinCPRS software program.

3.6 Statistical Analysis

For this dissertation the data collected from participants was averaged, and the mean differences were assessed with three statistical tools: t-tests, ANOVA, and correlation analysis.

Differences between two groups were assessed with t-tests, for groups of three or more a one-way repeated measures ANOVA (with a Bonferroni post-hoc test) was utilized, and associations between blood biomarkers and cardiovascular variables were assessed with correlation analysis (Pearson correlation). More specific details per variable are given in chapters 4 through 6, and appendix C of this dissertation shows the statistical analysis print outs for the primary outcome variables. T-tests and ANOVA were run in SigmaPlot, and correlation analysis was run in Microsoft Excel.

Changes in any variables labeled as an “increase” or “decrease” is referring to absolute differences among means. Means are presented along with the value of one standard deviation, and any p values derived from the statistical analyses are presented as absolute probability values with no one p value defined as a threshold value for statistical significance.

4 Results & Discussion (In-Depth Review)

The previous chapter described in detail the methodological undertakings within each experimental protocol that sought to explore the impact of food deprivation on cardiovascular physiology. This chapter, and the following two chapters, will present the findings of those experimental protocols, along with discussion on said findings. These three chapters are divided by experimental protocol category: in-depth review, acute food deprivation, and chronic food deprivation.

4.1 In-Depth Review Results

Pandemic contingency plan component four was an in-depth review of previously published research regarding the impact of acute and chronic food deprivation on cardiovascular physiology (specifically blood pressure and heart rate).

The initial PubMed search for articles was done in March of 2021 and it yielded 167 results. Filtering was then applied to exclude review articles, systematic reviews, and meta-analyses. This reduced the search down to 161 results. Further filtering was applied to only include articles written in English that focused on human adult participants. This further filtering reduced the search down to 83 results.

Each of these 83 results were individually assessed to determine whether or not they met the criteria of being an original research article that evaluated how food deprivation (acute or chronic) effected cardiovascular physiology (heart rate and/or blood pressure) in adult humans. This individual scrutinization yielded 19 individual articles, from the year

1988 to the year 2019. Additionally, after comparing this list of 19 articles with the list of 17 articles mentioned in chapter two (as a result of a similar PubMed search), the number of articles was increased by 7 to a total of 26 articles.

Thus, a total of 26 previously published research articles met the inclusion criteria for this in-depth review; 11 of these articles focused on acute food deprivation, and 15 of these articles focused on chronic food deprivation (Alam et al., 2019; Aliasghari, Izadi, Gargari, & Ebrahimi, 2017; Andersson, Wallin, Hedner, Ahlberg, & Andersson, 1988; Cansel et al., 2014; Chan et al., 2007; Dewanti, Watanabe, Sulistiawati, & Ohtsuka, 2006; Erdem et al., 2018; Harder-Lauridsen et al., 2017; Herbert et al., 2012; Hodgson, Burke, & Puddey, 2005; Horne et al., 2013; M'Guil et al., 2008; Mitchelmore, Stoner, Lambrick, Jobson, et al., 2018; Mitchelmore, Stoner, Lambrick, Sykes, et al., 2018; Mzoughi, Zairi, Jabeur, & Kraiem, 2018; Norouzy et al., 2017; Samad et al., 2015; Schulz et al., 2015; Seker et al., 2017; Shao et al., 2018; Solianik et al., 2016; Stoner et al., 2017; Sutton et al., 2018; Teng et al., 2013; Ural et al., 2008; Y. Young et al., 2015).

Because of the variety of differences among the articles (ex. experimental protocol, variables measured, participant population, etc) it was not possible or informative to perform one-to-one comparisons. Therefore, the blood pressure and heart rate data from the articles were extracted and compiled into a database for collective analysis. This method was informative, but there were limits to the analytics that could be performed. The results of this collective analysis are articulated below.

4.1.1 Acute Food Deprivation

Eleven of the in-depth review articles focused on acute food deprivation, 7 utilized healthy participants, and 4 utilized participants with one or more health conditions. The length of the acute food deprivation period lasted as short as 1 hour and as long as 72 hours. For this dissertation, only the data for healthy participants are shown (table 4.1).

Table 4.1: Acute food deprivation in-depth review articles

Article Title	Year Published	First Author	Protocol	# of Participants	Endpoints
Reliability of oscillometric central blood pressure and wave reflection readings: effects of posture and fasting	2015	Young	12-hour fast	20 (10F, 10M)	After a 12-hour fast; 45 min after eating
Reliability of pulse waveform separation analysis: effects of posture and fasting	2017	Stoner	12-hour fast	20 (10F, 10M)	After a 12-hour fast; 45 min after eating
Short-term food deprivation increases amplitudes of heartbeat- evoked potentials	2015	Schulz	18-hour fast	16F	After an 18-hour fast; 1 hour after eating
Effects of short-term food deprivation on interoceptive awareness, feelings and autonomic cardiac activity	2012	Herbert	24-hour fast	20F	After a 24-hour fast; 75 min after eating
Randomized cross-over trial of short-term water-only fasting: metabolic and cardiovascular consequences	2013	Horne	24-hour fast	30 (20F, 10M)	Before and after 24 hours of fasting; Before and after 24 hours of normal eating
Effect of 48 h fasting on autonomic function, brain activity, cognition, and mood in amateur weight lifters	2016	Solianik	48-hour fast	9M	After an 8-12 hr overnight fast; After a 48-hour fast
Short-term fasting-induced autonomic activation and changes in catecholamine levels are not mediated by changes in leptin levels in healthy humans	2007	Chan	72-hour fast	7F	After an overnight fast; After a 72-hour fast (+ an overnight fast)

The blood pressure and heart rate data organized by duration of acute food deprivation is presented below (table 4.2). The 'N' number refers to the number of articles for a particular data point. If more than one article presented the same data point (ex. HR after a 12-hour fast) then an average and standard deviation was generated from the multiple articles' data; otherwise, the average is just the mean data presented by the one article.

Table 4.2: Blood pressure and heart rate data for acute food deprivation protocols within in-depth review articles

Blood Pressure & Heart Rate (Healthy Participants; Acute Food Deprivation):

In-Depth Review

		Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (bpm)
After 1-hour Fast	Average	117.0	71.0	68.6
	Sd	-	-	3.64
	N	1	1	4
After 12-hour Fast	Average	113.6	67.7	66.6
	Sd	8.13	10.81	9.51
	N	3	3	4
After 18-hour Fast	Average	-	-	73.4
	Sd	-	-	-
	N	0	0	1
After 24-hour Fast	Average	110.9	68.9	71.3
	Sd	-	-	-
	N	1	1	1
After 48-hour Fast	Average	115.6	73.9	76.2
	Sd	-	-	-
	N	1	1	1
After 72-hour Fast	Average	107.7	56.2	69.4
	Sd	-	-	-
	N	1	1	1

Due to the limited number of articles with matching protocols, it was only possible to perform a statistical analysis for heart rate, comparing a 1-hour fast to a 12-hour fast. A non-paired t-test was used for this analysis with a resulting two-tailed p value of 0.712 .

4.1.2 Chronic Food Deprivation

Fifteen of the in-depth review articles focused on chronic food deprivation, 6 utilized healthy participants, 7 utilized participants with one or more health conditions, and 2 utilized a mix of healthy participants and participants with health conditions. The vast majority of the articles (13 out of 15) used Ramadan or an experimental protocol that mimicked Ramadan. For the two articles that did not use Ramadan, one did not detail the specifics of the protocol that was used, but the article alluded to possibly using Ramadan. The other non-Ramadan-using article utilized a protocol that involved two 13-hour fasts per week along with caloric restriction on the non-fasting days. For this dissertation, only the data from four articles for healthy participants participating in Ramadan were able to be utilized (table 4.3).

Ramadan is a religious practice that involves consuming no food or water during daylight hours, every day, for a one-month period of time. Typically, practitioners of Ramadan consume food and drink twice each day, once before sunrise and once after sunset. Ramadan is practiced once a year, during the month of Ramadan, as indicated by the Lunar Hijri calendar.

Table 4.3: Chronic food deprivation in-depth review articles

Article Title	Year Published	First Author	Protocol	# of Participants	Endpoints
Unexpected changes in blood pressure and hematological parameters among fasting and nonfasting workers during Ramadan in Indonesia	2006	Dewanti	Ramadan	100M	Pre-Iftar: 3 days before Ramadan; After the third week of Ramadan
The effects of Ramadan fasting on heart rate variability in healthy individuals: a prospective study	2014	Cansel	Ramadan	40 (16F, 24M)	24-hr EKG: Between the 13th and 17th days of Ramadan; During the first week after Ramadan
Effects of Ramadan fasting on blood pressure in normotensive males	2015	Samad	Ramadan	40M	Pre-Iftar & Post-Iftar during the week before Ramadan; During the 1st, 2nd & 3rd week of Ramadan; After Ramadan
Ramadan model of intermittent fasting for 28 d had no major effect on body composition, glucose metabolism, or cognitive functions in healthy lean men	2017	Harder-Lauridsen	Ramadan model (14-hr/d fast for 4 weeks)	10M	Before and after a 4-week control period; Before and after a 4-week treatment period

The blood pressure and heart rate data organized by point in time during Ramadan are presented below (table 4.4). The 'N' number refers to the number of articles for a

particular data point. If more than one article presented the same data point (ex. SBP before Ramadan) then an average and standard deviation was generated from the multiple articles' data; otherwise, the average is just the mean data presented by the one article.

Table 4.4: Blood pressure and heart rate data for chronic food deprivation protocols within in-depth review articles

Blood Pressure & Heart Rate (Healthy Participants; Chronic Food Deprivation):

In-Depth Review

		Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (bpm)
Before Ramadan	Average	127.9	79.4	82.4
	Sd	5.28	8.36	-
	N	3	3	1
Ramadan Week 1	Average	118.4	77.0	-
	Sd	-	-	-
	N	1	1	0
Ramadan Week 2	Average	120.5	74.3	78.0
	Sd	-	-	-
	N	1	1	1
Ramadan Week 3	Average	121.4	75.6	-
	Sd	3.68	1.98	-
	N	2	2	0
After Ramadan	Average	124.2	73.1	80.1
	Sd	-	-	-
	N	1	1	1

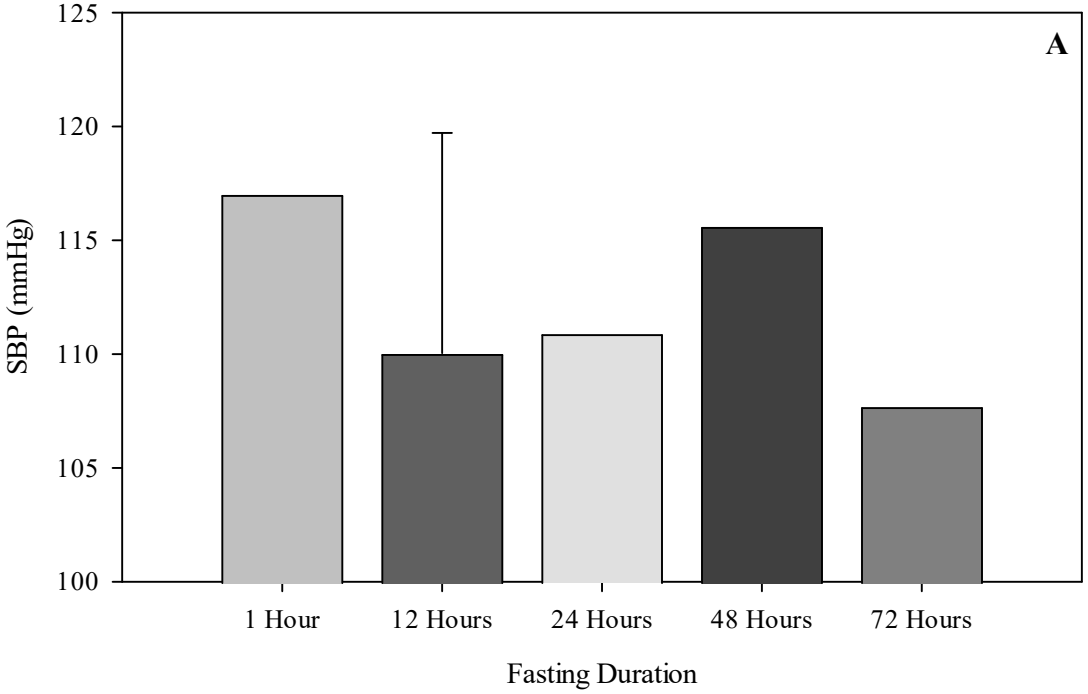
Due to the limited number of articles with matching protocols, it was only possible to perform a statistical analysis for blood pressure, comparing before Ramadan to Ramadan week three. Non-paired t-tests were used for this analysis with a resulting two-tailed p value of 0.233 for systolic blood pressure, and two-tailed a p value of 0.593 for diastolic blood pressure.

4.2 In-Depth Review Results Discussion

The following two sections provide a summary and discussion of the in-depth review results. Any error bars present on figures represent plus/minus one standard deviation unit.

4.2.1 Acute Food Deprivation

Regarding acute food deprivation, the collective results seem to indicate that blood pressure tends to decrease and heart rate tends to increase, over the course of a 72-hour period of food deprivation (figure 4.1). However, these changes were not simply linear changes over time.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105
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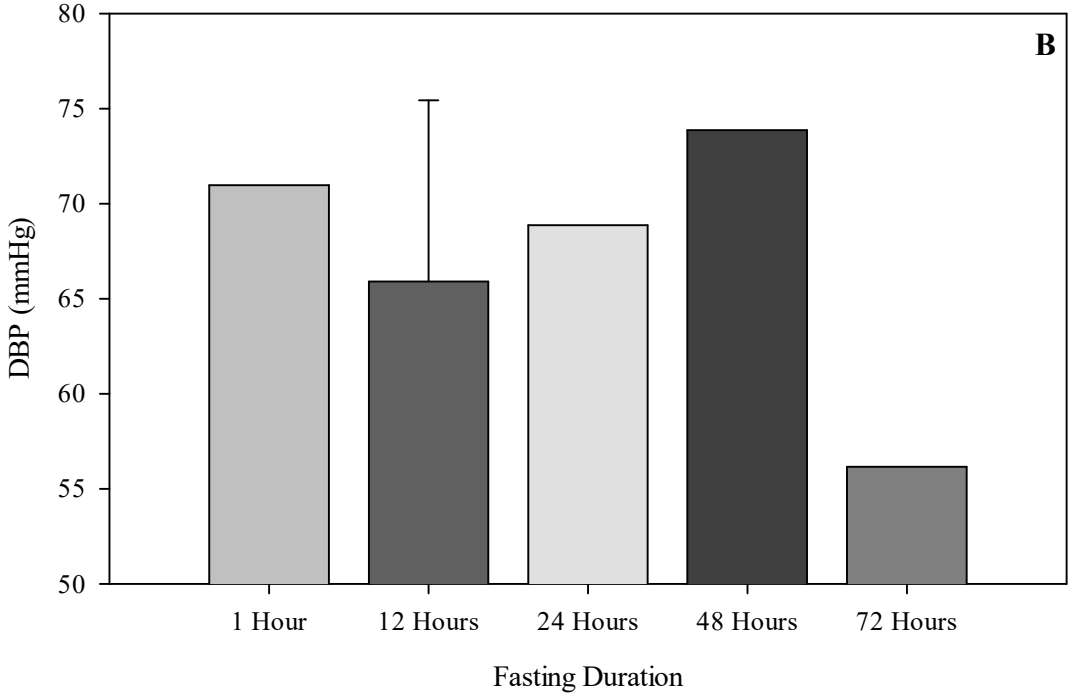
SBP (mmHg)
1 Hour
12 Hours
24 Hours
48 Hours
72 Hours
Fasting Duration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DBP (mmHg)
1 Hour
12 Hours
24 Hours
48 Hours
72 Hours
Fasting Duration

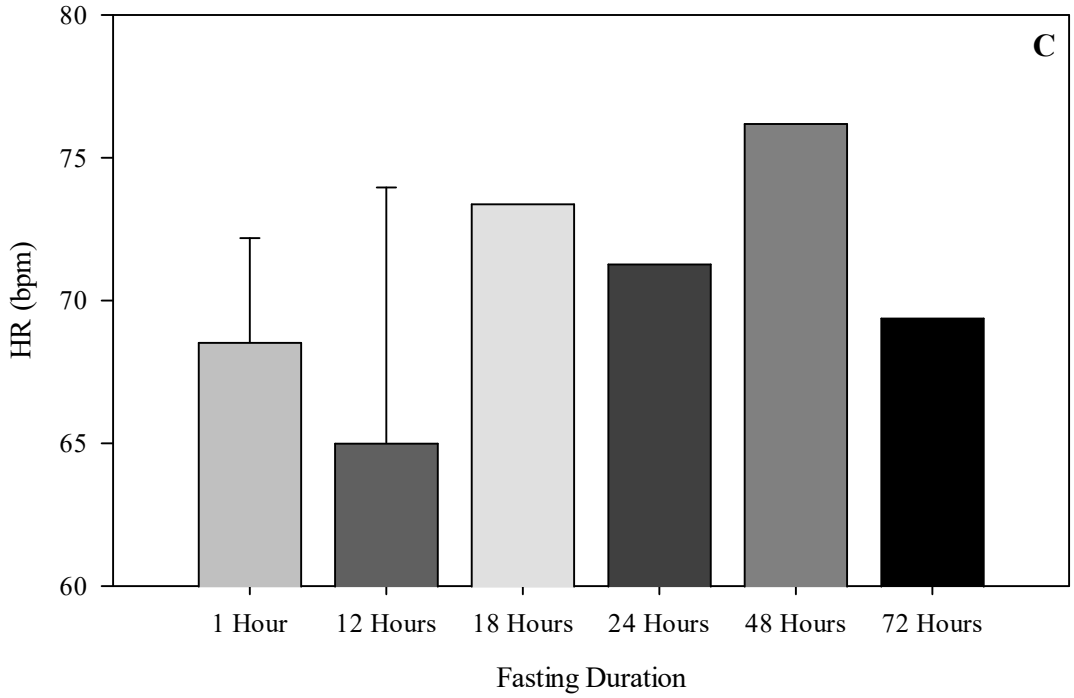


Figure 4.1: Systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C) combined data for acute food deprivation protocols from in-depth review articles

Systolic blood pressure, acutely, showed a general linear decrease from 1 hour post-prandial to 72 hours post-prandial. However, a very slight increase in SBP was shown at the 24-hour mark, relative to the 12-hour mark, and a larger increase in SBP at the 48-hour mark to just under the 1-hour mark level. Furthermore, the standard deviation value at the 12-hour mark brought it to above the 1-hour mark value. Lastly, the difference between the highest and lowest SBP values was approximately 10 mmHg.

Diastolic blood pressure, acutely, also showed a decrease when comparing the 1-hour post-prandial mark to the 72-hour post-prandial mark. However, the decrease in DBP at

the 12-hour mark was followed by an increase at the 24-hour and then 48-hour marks, with the 48-hour mark value being above the 1-hour post-prandial mark level. Furthermore, similar to SBP, the DBP standard deviation value at the 12-hour mark brought it to above both the 1-hour mark and 48-hour mark values. Lastly, the difference between the highest and lowest DBP values was approximately 17 mmHg.

Heart rate, acutely, showed an undulating increase from the 1-hour post-prandial mark to the 72-hour post-prandial mark. There was a decrease from the 1-hour mark to the 12-hour mark, and then an increase to the 18-hour mark, and then a decrease to the 24-hour mark, and then an increase to the 48-hour mark, and then finally a decrease to the 72-hour mark. All HR values for each mark were above the 1-hour mark level except for the 12-hour mark value. Furthermore, the standard deviation value for the 1-hour mark brought the HR value to above the 24-hour and 72-hour mark values, and the standard deviation value for the 12-hour mark brought it to above all values except for the 48-hour mark. And the p value of 0.712 gives only a 29% probability that the 1-hour mark and 12-hour mark HR values are statistically different due to the different fasting durations. Lastly, the difference between the highest and lowest HR values was approximately 10 bpm.

When comparing these in-depth review results (figure 4.1) to the summary of the previously published literature (table 2.1), there is not complete agreement between these two. For example, table 2.1 indicates that a 48-hour fast leads to a decrease in SBP, DBP and HR. But, figure 4.1 shows that SBP, DBP and HR are all at a higher value after a 48-hour fast compared to a 12-hour fast, and the 12-hour fasted state, in the morning, is generally considered the “gold standard” when measuring hemodynamic variables.

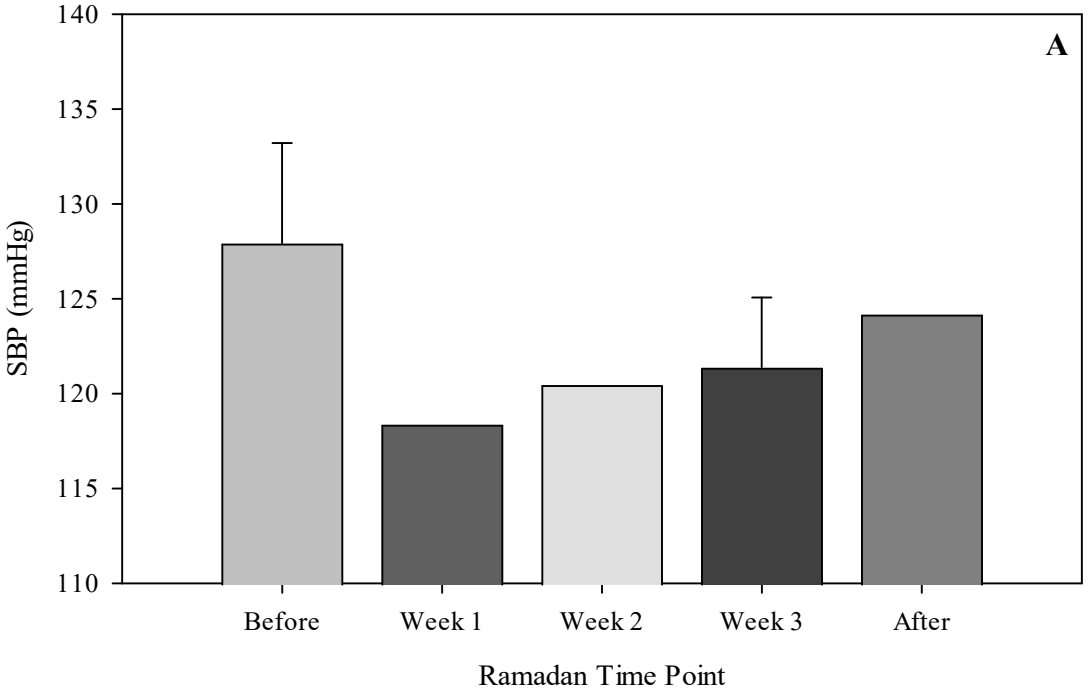
However, figure 4.1 also shows that the 48-hour fasted SBP value is less than the 1-hour fasted SBP value. This mismatch between table 2.1 and figure 4.1 can also be seen with a 12-hour fast, 24-hour fast and a 72-hour fast. And furthermore, according to figure 4.1, the SBP and DBP results differ for a 24-hour fast depending on whether a 1-hour fast or 12-hour fast is utilized as the baseline.

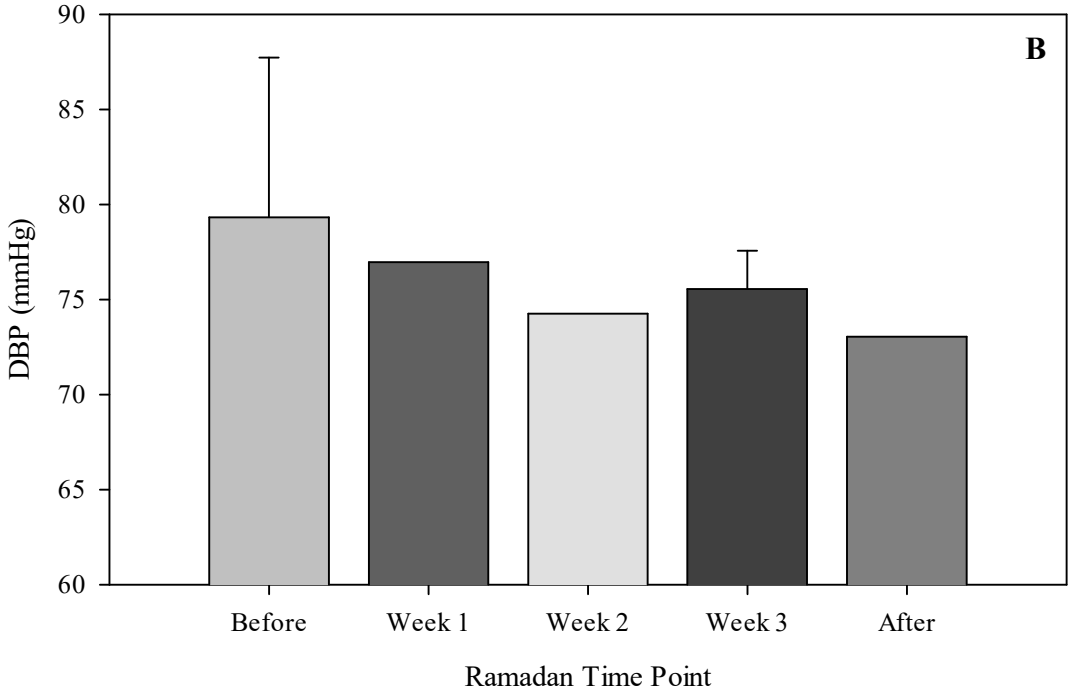
This mismatch between table 2.1 and figure 4.1 is possibly due to the seeming non-linear hemodynamic response to food deprivation, due to what is selected as the baseline condition to compare to food deprivation, and due to when hemodynamic measurements are taken.

One important question derived from this is: "what is considered baseline with respect to food deprivation?". Is baseline considered the moment immediately following food consumption? Or is baseline considered to be after someone's food has "settled"? Or perhaps baseline is considered to be first thing in the morning after the overnight fasting period? This determination of baseline is important because what is utilized as baseline will have an influence on what kind of change, and to what magnitude, will be interpreted from acute food deprivation. And furthermore, a clearer understanding of acute food deprivation will better aid in understanding chronic food deprivation.

4.2.2 Chronic Food Deprivation

Regarding chronic food deprivation, the collective results seem to indicate a decrease in blood pressure and heart rate throughout the chronic food deprivation period (figure 4.2). However, again, these changes were not simply linear changes over time.


A
140
135
130
125
120
115
110
SBP (mmHg)
Before
Week 1
Week 2
Week 3
After
Ramadan Time Point


B
90
85
80
75
70
65
60
DBP (mmHg)
Before
Week 1
Week 2
Week 3
After
Ramadan Time Point

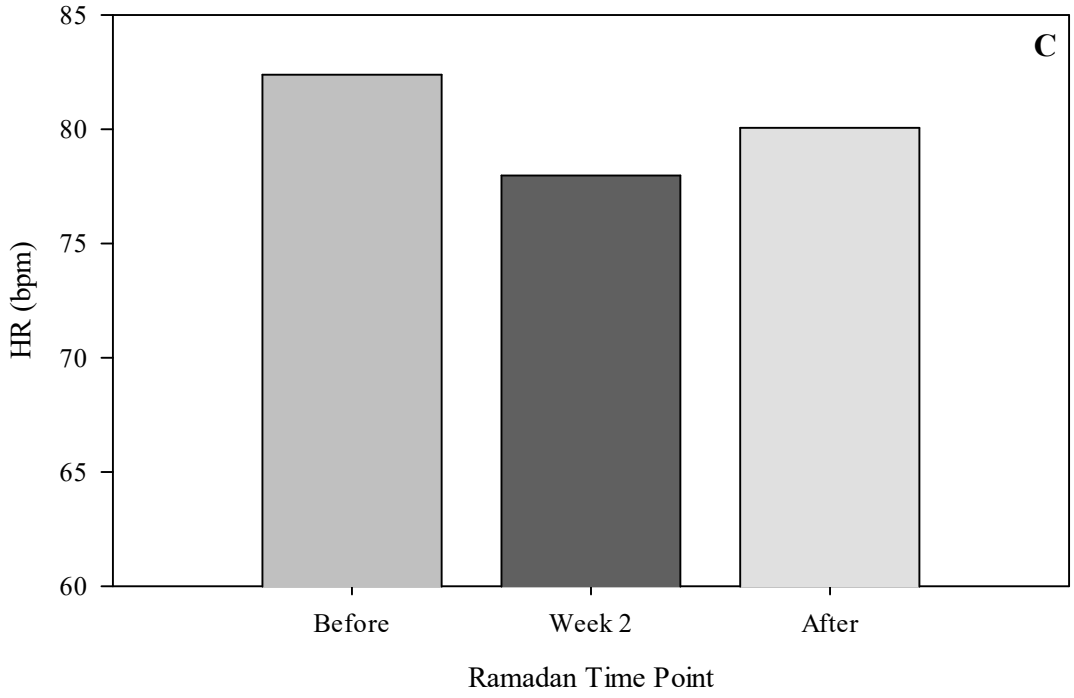


Figure 4.2: Systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C) combined data for chronic food deprivation protocols from in-depth review articles

Systolic blood pressure, chronically, showed to be lower throughout and after Ramadan, compared to before Ramadan. The lowest SBP value occurred during week one of Ramadan and the systolic blood pressure values increased linearly from week one to after Ramadan. Furthermore, the week three SBP standard deviation brought the week three value to above the after Ramadan value. And the p value of 0.233 gives a 77% probability that the before Ramadan and week three of Ramadan SBP values are statistically different due to their time point in Ramadan. Lastly, the difference between the highest and lowest SBP values was approximately 10 mmHg.

Diastolic blood pressure, chronically, showed a mostly linear pattern, with a decrease in the DBP value from before Ramadan to After Ramadan. However, there was a slight increase at week three of Ramadan, relative to week two, but all values throughout and after Ramadan were lower than before Ramadan. Furthermore, the week three DBP standard deviation brought the week three value to above all other values except for the before Ramadan DPB value. And the p value of 0.593 gives a 41% probability that the before Ramadan and week three of Ramadan DBP values are statistically different due to their time point in Ramadan. Lastly, the difference between the highest and lowest DBP values was approximately 6 mmHg.

Heart rate, chronically, only had values for before Ramadan, week two of Ramadan, and after Ramadan. The before Ramadan value was the highest, followed by after Ramadan, and then week two of Ramadan. Lastly, the difference between the highest and lowest HR values was approximately 4 bpm.

When comparing these in-depth review results (figure 4.2) to the summary of the previously published literature (table 2.2), there is mostly complete agreement between these two. The one point of disagreement comes from a study in 2017 by Seker et al that indicates increased SBP and DBP in those participating in Ramadan compared to those not participating in Ramadan (Seker et al., 2017). This study took hemodynamic measurements for 24 hours, but only once for each participant in each group, and participants were not measured at the same time point of Ramadan.

This mostly agreement between table 2.2 and figure 4.2 is likely due to their being a minimal number of chronic food deprivation studies along with the vast majority of those studies utilizing the same protocol, Ramadan.

4.3 Summary

The in-depth review results seem to indicate that there is generally a uniform overall cardiovascular response to chronic food deprivation, and a non-linear cardiovascular response throughout an extending period of acute food deprivation. Furthermore, the hemodynamic response to food deprivation, although seemingly very present, is relatively mild compared to other stressors, such as exercise.

The next two chapters of this dissertation will delve into the results of the acute and chronic food deprivation protocols within this dissertation research project and relate them back to this in-depth review as well as the previously published literature.

5 Results & Discussion (Acute Food Deprivation)

The previous chapter presented the results of the in-depth review along with a discussion of said results. This chapter, and the following chapter, will present the findings of the experimental protocols that focused on acute or chronic food deprivation, along with discussion on said findings. This chapter, specifically, will focus on acute food deprivation, and includes data compiled from the original protocol, pandemic contingency plan component one, and pandemic contingency plan component two.

5.1 Recruitment Overview

Recruitment for this research project began in January of 2020 and continued, intermittently, as needed, until March of 2021. Over this course of time a total of 101 individuals responded to recruitment materials for this research project. Of these respondents, 71% were responsive to communication after initially expressing interest. Of the responsive respondents, 11% were uninterested in participating, 40% were unable or ineligible, and 49% were enrolled in one of the protocols.

Of the total number of enrolled participants, 9% dropped out, 43% were unable to complete data collection due to COVID-19 pandemic restrictions, and 48% did complete data collection. Thus, out of the 101 initial respondents, 35 were enrolled in one of the protocols, and 17 did fully complete data collection.

5.2 Data Collection Overview

Data collection for this research project took place from February of 2020 until May of 2021, with a variety of starts and stops along the way, due to COVID-19 pandemic restrictions. More details are given in the sections below.

5.2.1 Original Protocol

Data collection for the original protocol began on February 11, 2020 and was able to continue until March 18, 2020. At this point, all in-person research activity was stopped at Michigan Technological University due to COVID-19 pandemic restrictions. This cessation, or pause, of in-person research activity for this research project continued from March of 2020 until September of 2020. The timing and length of this in-person research activity shutdown did not allow any of the participants enrolled in the original protocol to complete the full data collection protocol.

There were a total of 16 participants enrolled in the original protocol. Two participants dropped out before beginning data collection, and one participant was unable to begin data collection due to research activity being shut down prior to their first testing session. Therefore, 13 participants were able to attend at least one testing session, but no participants were able to attend all eight testing sessions.

The specific breakdown of testing sessions completed by participants were as follows: 3 participants completed 0 testing sessions, 2 participants completed 2 testing sessions, 8

participants completed 3 testing sessions, and 3 participants completed 5 testing sessions. No participants were able to complete more than 5 testing sessions.

The first three testing sessions occurred in the control condition only, so only 3 participants were able to generate data for both the control and treatment condition. Therefore, the ambulatory acute phase data (control and treatment) along with the data from testing sessions one, two, four, and five for these 3 participants were procured for analysis. The remaining data for all original protocol participants were considered unusable at this time.

5.2.2 Pandemic Contingency Plan Component One

Data collection for the pandemic contingency plan component one protocol began on October 7, 2020 and was able to continue until November 15, 2020. At this point, all in-person research activity was once again stopped at Michigan Technological University due to COVID-19 pandemic restrictions. This second cessation, or pause, of in-person research activity for this research project continued from November of 2020 until February of 2021. The timing and length of this second in-person research activity shutdown did not allow any of the participants enrolled in the pandemic contingency plan component one protocol to complete data collection.

Approval was granted for up to two participants for this protocol. Only one participant was enrolled in the pandemic contingency plan component one protocol. A second individual was interested in participating but was ultimately unable to participate.

The one participant enrolled in this protocol was only able to complete 5 testing sessions before the in-person research activity shutdown occurred. Similar to the original protocol, the ambulatory acute phase data (control and treatment) along with the data from testing sessions one, two, four, and five for this participant were procured for analysis. The remaining data were considered unusable at this time.

5.2.3 Pandemic Contingency Plan Component Two

Data collection for the pandemic contingency plan component two protocol began on September 28, 2020 and continued until November 13, 2020. The first and second suspensions of in-person research activity occurred before data collection began for this protocol and after data collection ended for this protocol. Thus, the in-person research activity suspensions did not affect data collection for this protocol.

Approval was granted to complete data collection for up to 11 participants for this protocol. There were a total of 12 participants enrolled in the pandemic contingency plan component two protocol; 1 participant dropped out after two testing sessions, and the remaining 11 participants completed all four testing sessions. However, one of the 11 participant's data were excluded due to the participant not adhering to all the stipulations of the experimental conditions. This resulted in this protocol having complete, usable data for 10 participants.

Furthermore, the 4 participants' data from the original protocol and the pandemic contingency plan component one protocol were joined with the data from the pandemic

contingency plan component two protocol. This conjoining of data created an acute food deprivation dataset that consisted of 14 total participants (6 female / 8 male).

5.3 Acute Food Deprivation Experimental Protocols

As a reminder, and reference, the following figures display the experimental timeline for the original protocol (figure 5.1), the pandemic contingency plan component one protocol (figure 5.1), and the pandemic contingency plan component two protocol (figure 5.2).

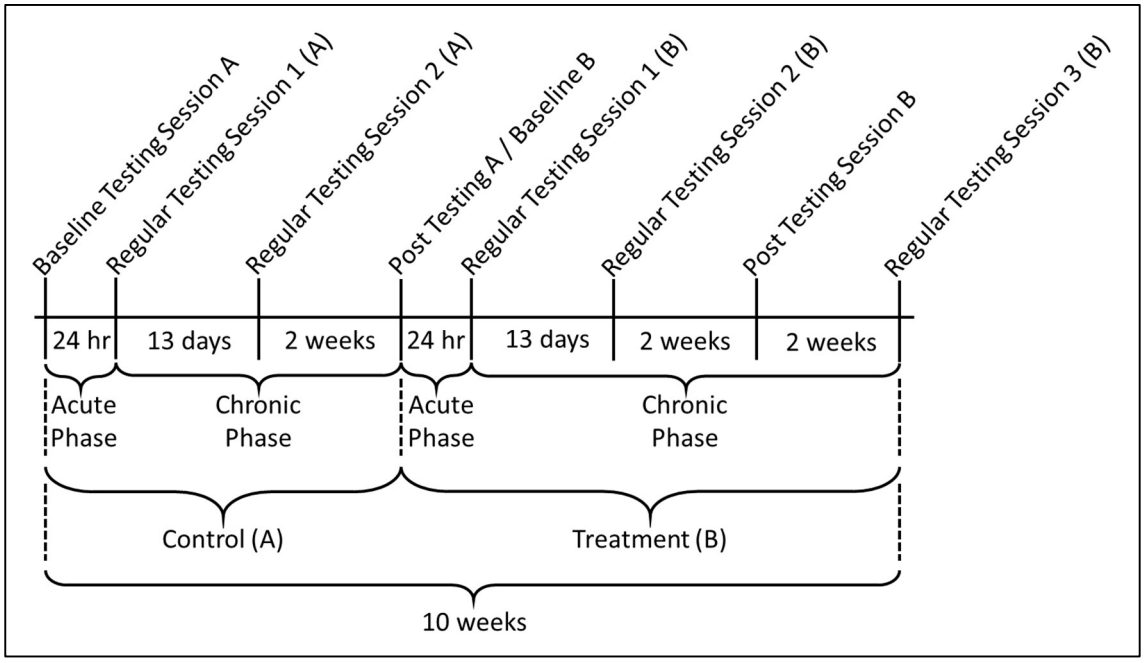


Figure 5.1: Experimental timeline of original protocol and pandemic contingency plan component one protocol

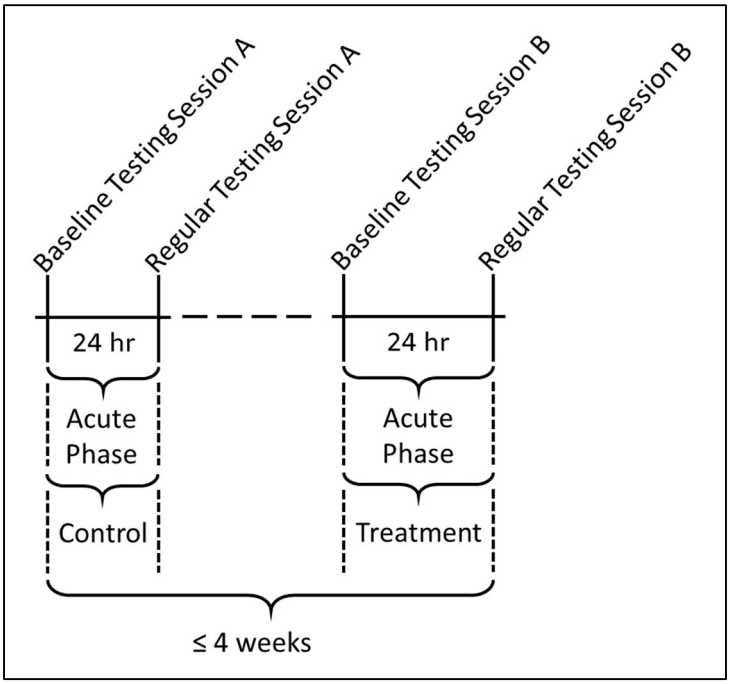


Figure 5.2: Experimental timeline of pandemic contingency plan component two protocol

Regardless of protocol, each participant completed four visits for the acute food deprivation data. Visits 1 and 2 were at the beginning and end of the 24-hour normal food consumption (control) period. Visits 3 and 4 were at the beginning and end of the 24-hour food deprivation (treatment) period. In addition to the four visits, each participant also had their ambulatory blood pressure and heart rate monitored throughout each (control and treatment) 24-hour period.

5.4 Acute Food Deprivation Results

The acute food deprivation results are organized below.

5.4.1 Demographics and Lifestyle Characteristics

The average age of the participants was 26 years with a standard deviation of 8.62 years. Regarding identity, participants gave 26 total answers; 42% of answers fit within the classification category, 54% fit within the personality category, and 4% fit within the appearance category.

For the female participants, the average menstrual cycle phase was day 7.8 (visit 1 = day 3.2±0.75; visit 2 = day 4.2±0.75; visit 3 = day 11.3±9.81; visit 4 = day 12.3±9.81). The goal for the acute food deprivation protocols was to have female participants come to their lab visits within the first week (days 1-7) of their menstrual cycle. Only 7 of the 24 total visits, for all female participants, occurred outside of this first week time frame, with 5 of those 7 visits occurring no later than the 11^{th} day of the menstrual cycle. There was one participant whose menstrual cycle exceeded 28 days, and since visits two and three occurred four weeks apart for this participant, this led to visits three and four occurring on the 31^{st} and 32^{nd} day of the menstrual cycle, respectively.

The average amount of sleep, for all participants, was 7.9±1.10 hours during the control period and was 8.2±1.33 hours during the treatment period. The average pre-visit fasting duration for visits 1-3 was 10.9 hours (visit 1 = 11.0±2.95 hours; visit 2 = 10.8±2.59 hours; visit 3 = 11.0±2.84 hours). The average pre-visit fasting duration was 23.3±0.54 hours for visit four. Regarding the pre-visit fasting duration data, for visits one, two, and three, participants were asked to finish consuming food at least three hours prior to their

visit and were told that finishing eating the night before their visit was acceptable. Visit four occurred at the end of the 24-hour acute fasting period.

In regard to physical activity level, the average fitness level of participants was 2.3±0.73, and the average change in physical activity level was -0.1±0.53 from the control period visits to the treatment period visits. And regarding stress level, the average total stress of participants was 4.1±2.51, and the average change in stress level was 0.0±0.55 from the control period visits to the treatment period visits.

5.4.2 Anthropometrics

The baseline anthropometric data are shown in the table (table 5.1) below and were measured during the first laboratory visit.

Table 5.1: Baseline anthropometric data for acute food deprivation participants

Anthropometrics:

Acute Food Deprivation

		Baseline Measurements
Height (m)	Average Sd	1.72 0.07
Mass (kg)	Average Sd	76.6 13.80
Fat Mass (%)	Average Sd	27.5 7.67
Bone Mineral Content (g)	Average Sd	2701.2 409.18
Bone Mineral Density (g/cm^2)	Average Sd	1.21 0.11

The only anthropometric variable that was measured during all four visits and showed a change, was mass. The change in mass during the control period (visit 1 to visit 2) was 0.12±0.67 kg, and the change in mass during the treatment period (visit 3 to visit 4) was -1.31±0.58 kg. A two-tailed p value of 0.00002 was calculated from a paired t-test comparing the change in mass between the control and treatment periods.

5.4.3 Hemodynamics

The hemodynamic data are shown in the tables below for seated rest (table 5.2), change over 24 hours (table 5.3), and the 24-hour ambulatory measurements (table 5.4).

Table 5.2: Seated rest hemodynamic data for acute food deprivation participants

Hemodynamics (Resting Values):

Acute Food Deprivation

		Visit 1	Visit 2	Visit 3	Visit 4
Systolic Blood Pressure (mmHg)	Average	112.4	109.6	110.6	111.6
	Sd	12.31	12.17	12.56	13.57
Diastolic Blood Pressure (mmHg)	Average	68.9	67.1	67.3	67.6
	Sd	10.70	8.73	9.92	9.05
Heart Rate (bpm)	Average	72.8	73.6	71.9	74.9
	Sd	12.47	14.08	11.53	11.65

Table 5.3: Change over 24 hours (calculated from seated rest data) hemodynamic data for acute food deprivation participants

Hemodynamics (Change Over 24 Hours):

Acute Food Deprivation

		24-hr Change (control)	24-hr Change (treatment)	p value
Systolic Blood Pressure (mmHg)	Average	-2.79	0.93	0.062
	Sd	8.36	4.75	
Diastolic Blood Pressure (mmHg)	Average	-1.86	0.36	0.101
	Sd	5.10	2.92	
Heart Rate (bpm)	Average	0.79	3.00	0.125
	Sd	7.39	5.68	

Table 5.4: 24-hour ambulatory hemodynamic data for acute food deprivation participants

Hemodynamics (Ambulatory Values):

Acute Food Deprivation

		Control	Treatment	p value
24-hr Total Ambulatory Systolic Blood Pressure (mmHg)	Average	119.7	118.1	0.159
	Sd	7.70	8.87	
24-hr Awake Ambulatory Systolic Blood Pressure (mmHg)	Average	124.8	122.7	0.120
	Sd	8.49	9.45	
24-hr Asleep Ambulatory Systolic Blood Pressure (mmHg)	Average	109.3	109.4	0.460
	Sd	8.20	8.87	
24-hr Total Ambulatory Diastolic Blood Pressure (mmHg)	Average	68.8	67.6	0.167
	Sd	5.30	6.46	
24-hr Awake Ambulatory Diastolic Blood Pressure (mmHg)	Average	74.3	72.7	0.161
	Sd	4.90	6.13	
24-hr Asleep Ambulatory Diastolic Blood Pressure (mmHg)	Average	57.7	58.1	0.407
	Sd	6.69	7.60	
24-hr Total Ambulatory Heart Rate (bpm)	Average	68.3	66.3	0.076
	Sd	8.01	6.95	
24-hr Awake Ambulatory Heart Rate (bpm)	Average	71.9	69.4	0.062
	Sd	8.80	8.11	
24-hr Asleep Ambulatory Heart Rate (bpm)	Average	60.3	60.3	0.484
	Sd	8.83	6.16	

Paired t-tests were used to calculate the one-tailed p values shown in the previous tables.

5.4.4 Blood Biomarkers

The blood biomarkers data are shown in the tables below for seated rest (table 5.5) and for change over 24 hours (table 5.6).

Table 5.5: Seated rest blood biomarkers data for acute food deprivation participants

Blood Biomarkers (Resting Values):

Acute Food Deprivation

		Visit 1	Visit 2	Visit 3	Visit 4
Blood Glucose (mg/dl)	Average	95.8	95.1	94.9	86.4
	Sd	13.32	11.37	9.13	12.77
Plasma Ghrelin (pg/ml)	Average	185.26	170.13	146.91	180.69
	Sd	156.80	151.81	125.56	210.07
Plasma LEAP2 (ng/ml)	Average	0.82	0.13	0.75	0.41
	Sd	1.88	0.91	1.44	1.24
Plasma NPY (pg/ml)	Average	866.21	797.06	890.52	432.28
	Sd	626.06	653.04	742.39	547.56

Table 5.6: Change over 24 hours blood biomarkers data for acute food deprivation participants

Blood Biomarkers (Change Over 24 Hours):

Acute Food Deprivation

		24-hr Change (control)	24-hr Change (treatment)	p value
Blood Glucose (mg/dl)	Average	-0.64	-8.50	0.012
	Sd	9.69	7.23	
Plasma Ghrelin (pg/ml)	Average	-15.13	33.78	0.171
	Sd	36.55	164.28	
Plasma LEAP2 (ng/ml)	Average	-0.71	-0.31	0.203
	Sd	1.51	1.83	
Plasma NPY (pg/ml)	Average	-88.90	-493.26	0.007
	Sd	245.64	439.41	

Paired t-tests were used to calculate the one-tailed p values in the previous table. Furthermore, one male participant's plasma ghrelin data and one male participant's plasma LEAP2 data were excluded due to a lack of both control and treatment data to compare.

5.4.5 Correlations

Correlation analysis was used to assess the relationship between changes in hemodynamics and changes in blood biomarkers during the treatment period as well as

changes amongst the blood biomarkers themselves. The results of these correlation analyses are shown in the table below (table 5.7).

Table 5.7: Correlation analyses results for acute food deprivation participants

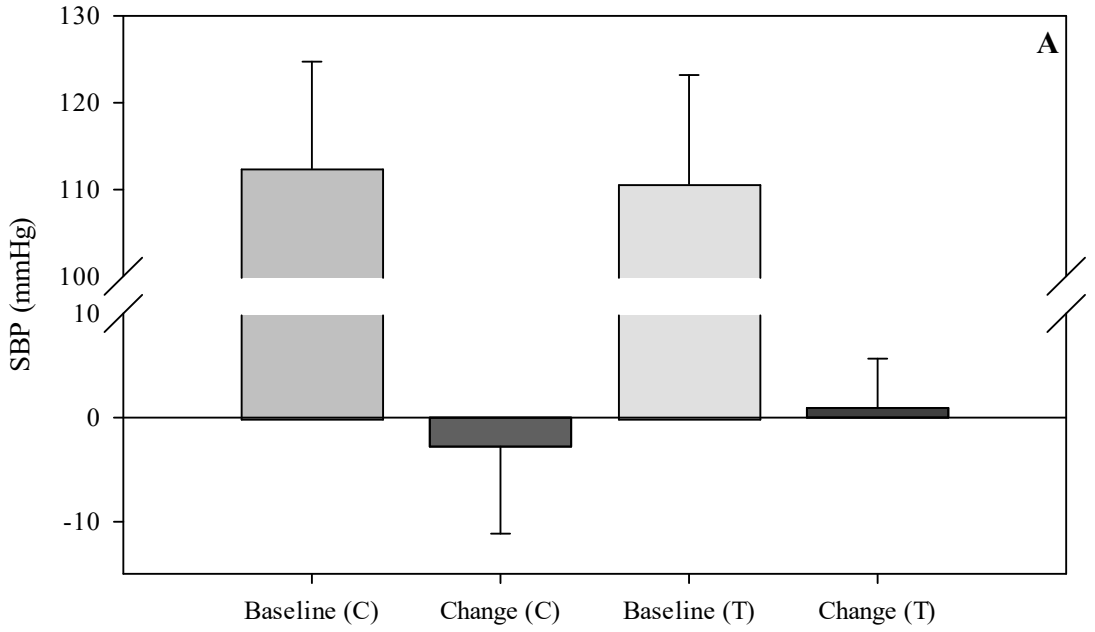
Correlations:

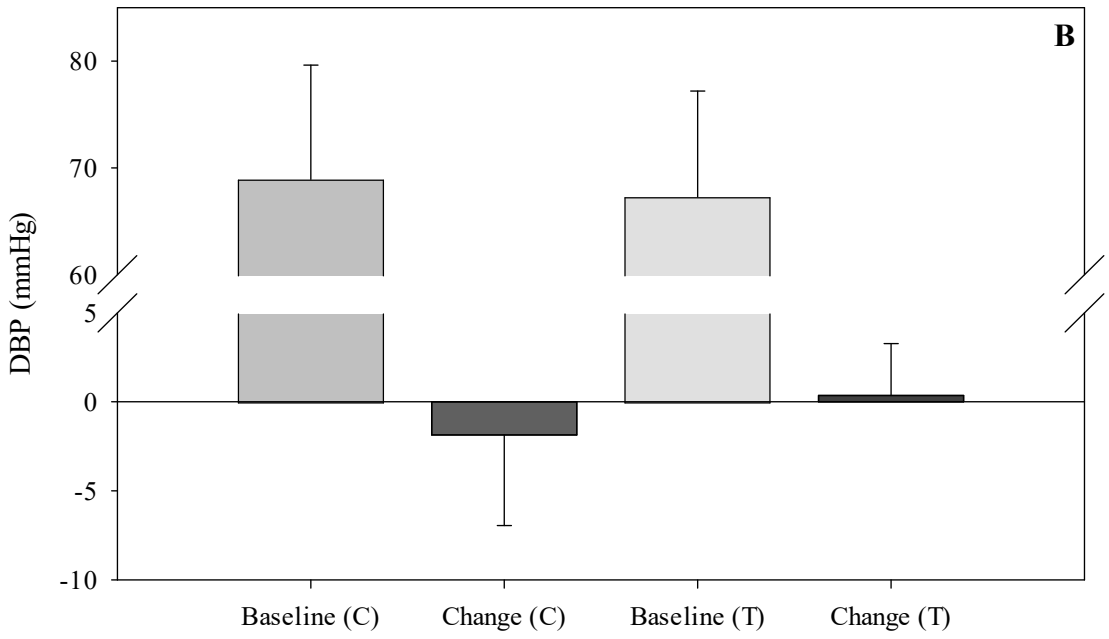
Acute Food Deprivation

	r	r^2
SBP ↔ Glucose	0.37	0.14
SBP ↔ Ghrelin	-0.13	0.02
SBP ↔ LEAP2	-0.10	0.01
SBP ↔ NPY	-0.06	0.003
DBP ↔ Glucose	-0.14	0.02
DBP ↔ Ghrelin	0.39	0.15
DBP ↔ LEAP2	-0.003	0.000009
DBP ↔ NPY	0.24	0.06
HR ↔ Glucose	-0.44	0.19
HR ↔ Ghrelin	-0.19	0.04
HR ↔ LEAP2	-0.20	0.04
HR ↔ NPY	-0.22	0.05
Glucose ↔ Ghrelin	-0.36	0.13
Glucose ↔ LEAP2	-0.25	0.07
Glucose ↔ NPY	-0.23	0.05

5.5 Acute Food Deprivation Results Discussion

Regarding the cardiovascular response to the acute food deprivation protocol for this research project, the pre and post 24-hour fast data showed increased systolic and diastolic blood pressure as well as heart rate after 24 hours of acute food deprivation, with less variation (standard deviation) in the 24-hour change data during the treatment condition compared to the control condition (figure 5.3). Additionally, the 24-hour average ambulatory data showed a decrease in these same variables (SBP, DBP, HR), when considering treatment compared to control data, and this decrease in ambulatory hemodynamic variables during the treatment condition only showed for the overall and awake data and not for the during sleep data (figure 5.4). Lastly, the greatest amount of change for both the pre/post 24-hour and ambulatory data occurred with HR compared to SBP and DBP.


A
SBP (mmHg)
130
120
110
100
10
0
-10
Baseline (C)
Change (C)
Baseline (T)
Change (T)


B
DBP (mmHg)
80
70
60
5
0
-5
-10
Baseline (C)
Change (C)
Baseline (T)
Change (T)

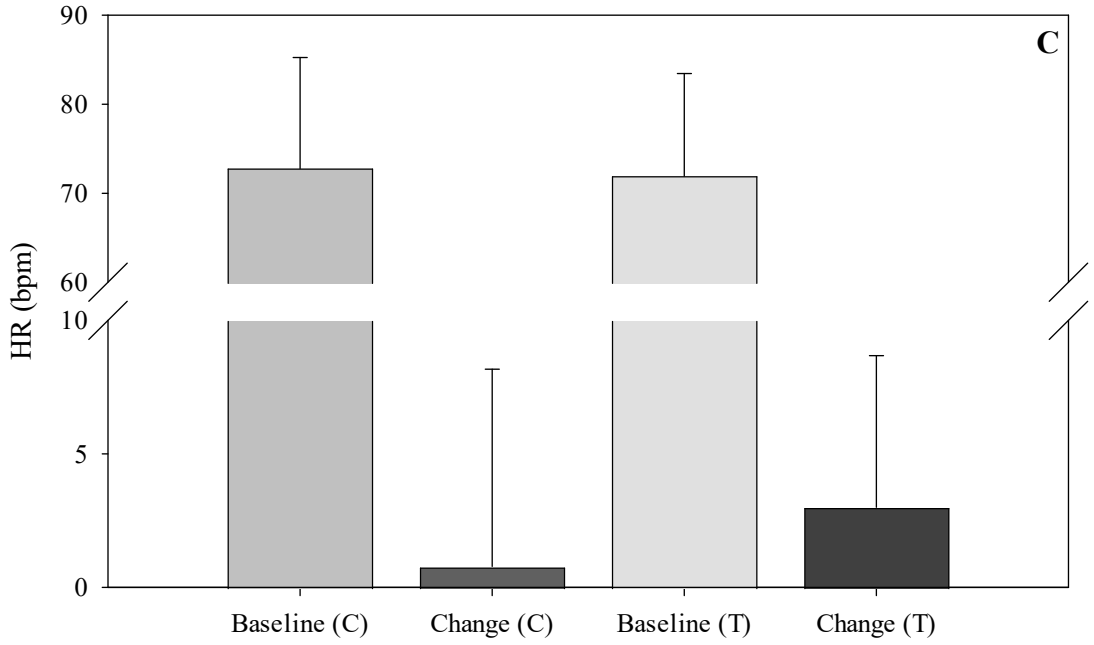
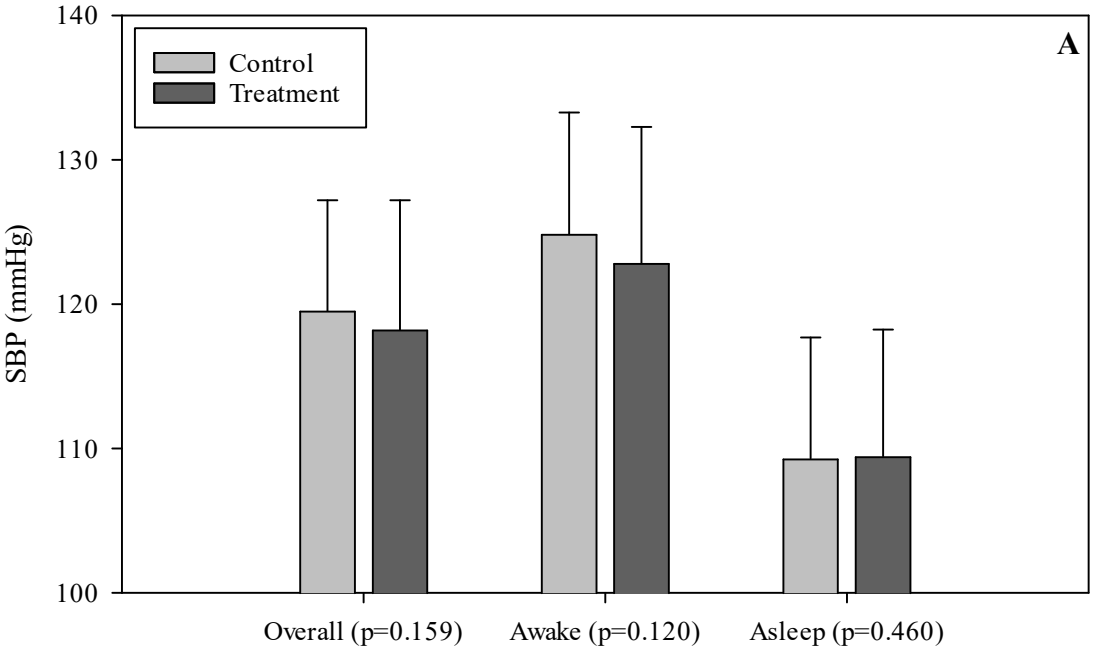
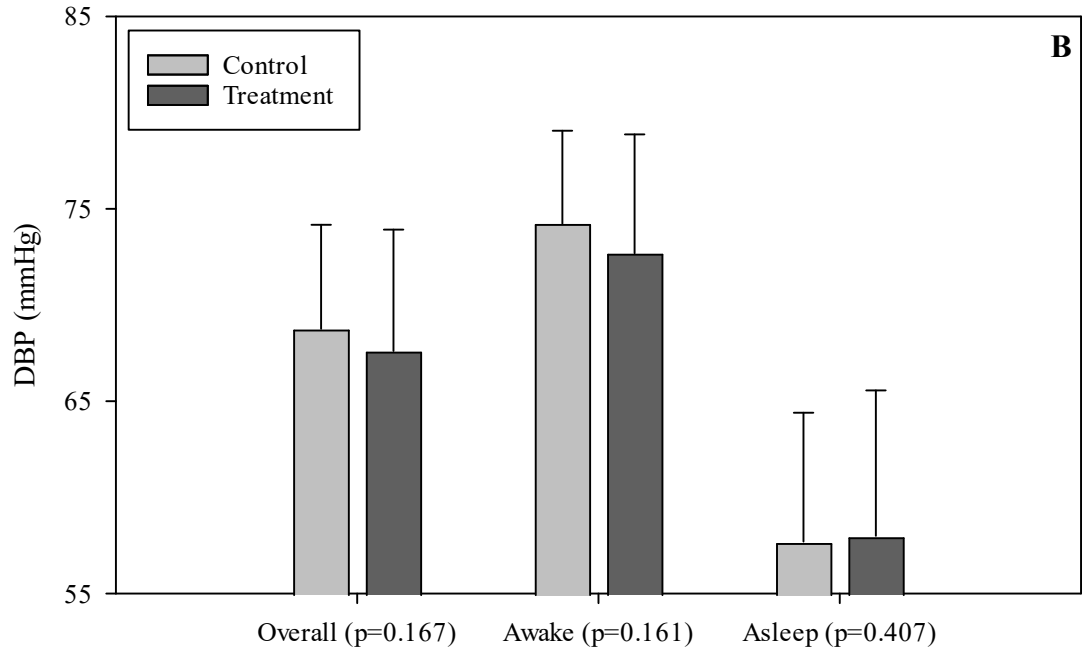


Figure 5.3: Change over 24 hours in resting systolic blood pressure (A; baseline p=1.000 & change p=0.062), diastolic blood pressure (B; baseline p=1.000 & change p=0.101) and heart rate (C; baseline p=1.000 & change p=0.125) for acute food deprivation participants; 'C' = control and 'T' = treatment


A
Control
Treatment
SBP (mmHg)
140
130
120
110
100
Overall (p=0.159)
Awake (p=0.120)
Asleep (p=0.460)


B
Control
Treatment
DBP (mmHg)
85
75
65
55
Overall (p=0.167)
Awake (p=0.161)
Asleep (p=0.407)

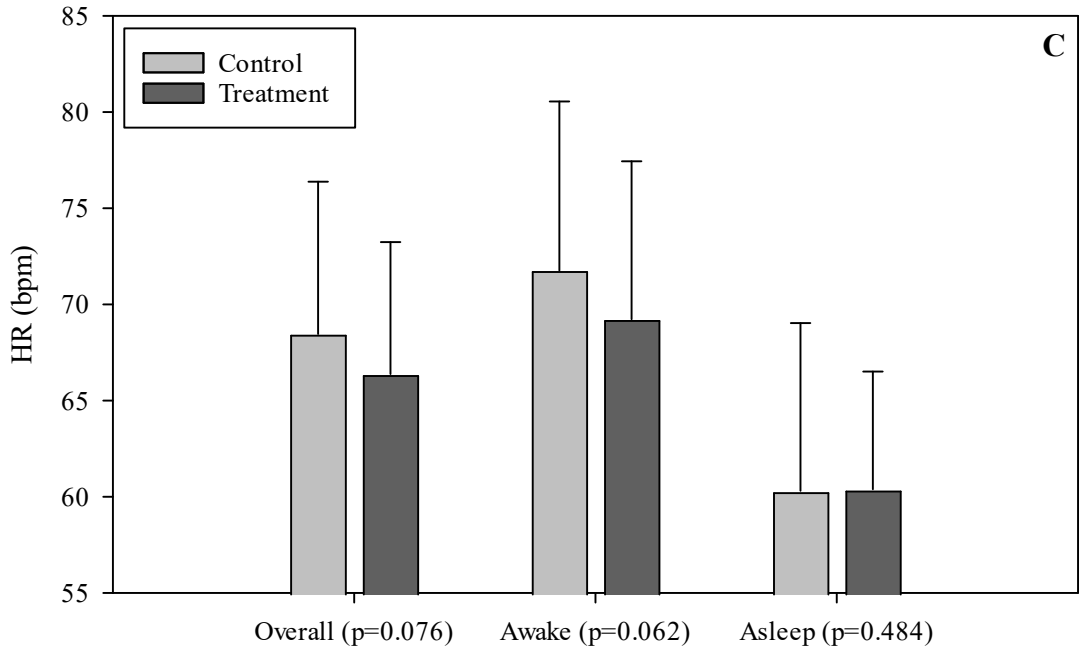
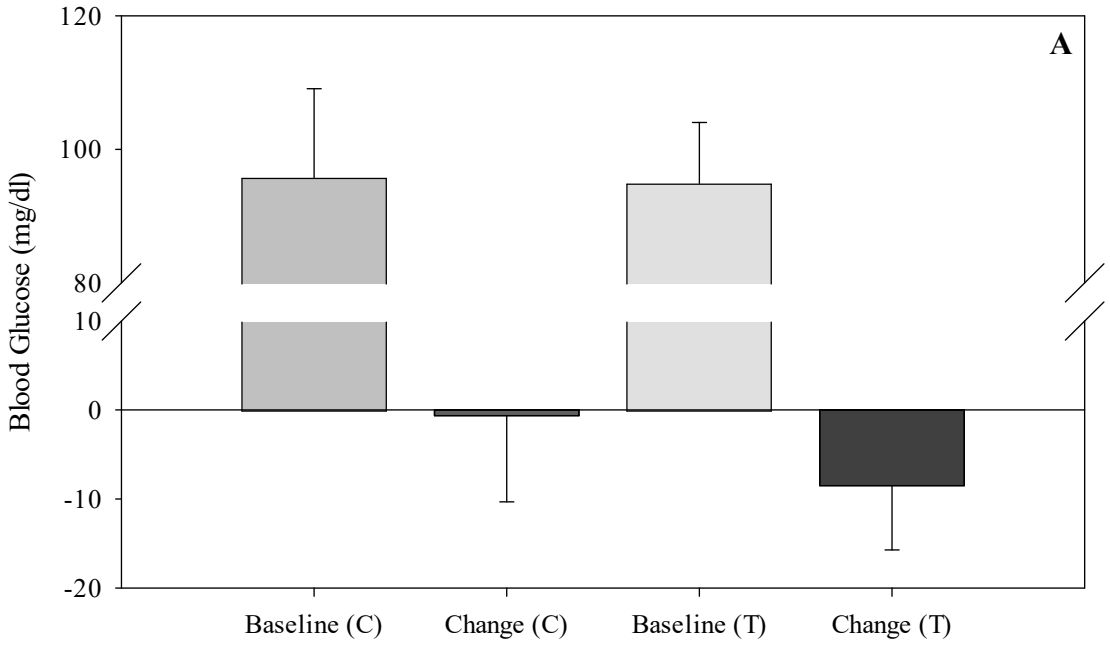


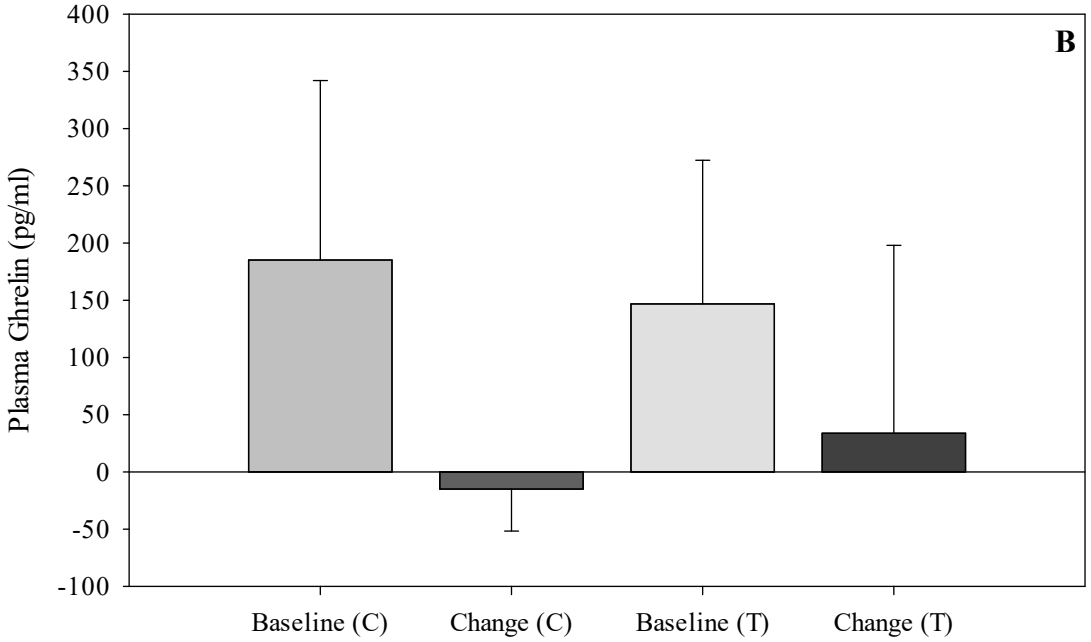
Figure 5.4: Average ambulatory systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C) throughout 24 hours for acute food deprivation participants

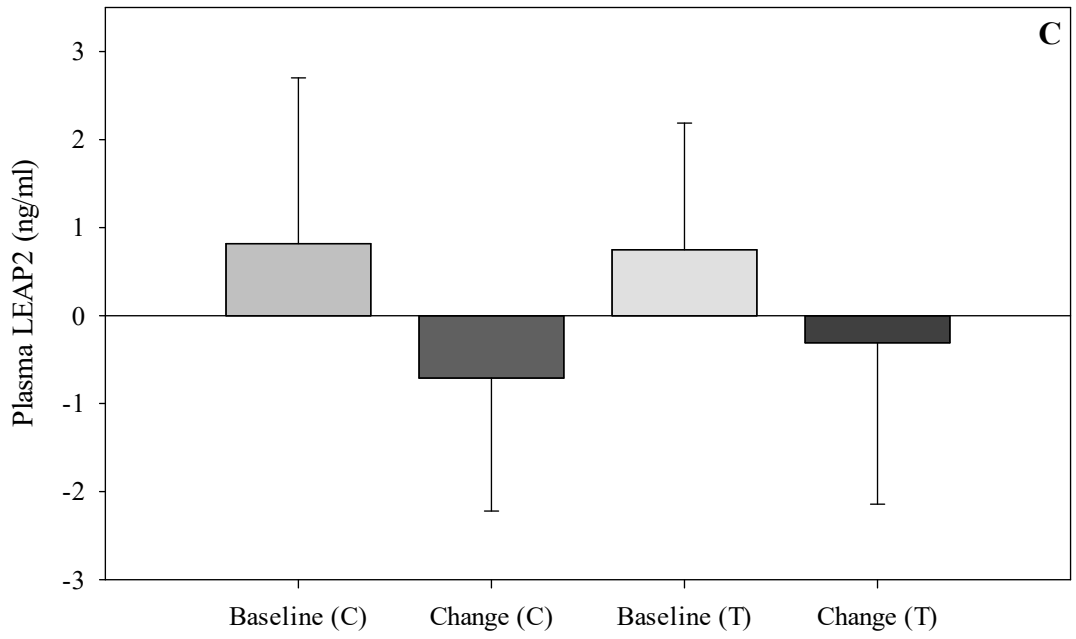
The acute food deprivation hemodynamic results of this research project are generally in line with other data. Previously published data (table 2.1) have shown that a 24-hour fast can lead to increased DBP (Horne et al., 2013), and increased HR along with decreased HRV (Herbert et al., 2012). Additionally, the in-depth review data (figure 4.1) also show that a 24-hour fast can lead to increased SBP, DBP and HR when compared to a 12-hour fast. Lastly, another dissertation research project from the same laboratory, that also studied the effects of a 24-hour acute fast, found there to be lower 24-hour average ambulatory SBP, DBP and HR while fasting (Gonzalez, 2021; Gonzalez, Stelly, & Cooke, 2021).

However, although the acute food deprivation hemodynamic results of this research project are in line with most other data, they are not in line with all other data. Horne et al (Horne et al., 2013) found decreased SBP after a 24-hour fast and the other dissertation mentioned above (Gonzalez, 2021) found increased RRI during controlled breathing after a 24-hour fast.

Along with these hemodynamic changes, the acute food deprivation protocol for this research project also showed changes in blood biomarkers after a 24-hour acute fast: decreased blood glucose, increased plasma ghrelin, increased plasma LEAP2 and decreased plasma NPY (figure 5.5).




B
Plasma Ghrelin (pg/ml)
400
350
300
250
200
150
100
50
0
-50
-100
Baseline (C)
Change (C)
Baseline (T)
Change (T)


C
Plasma LEAP2 (ng/ml)
3
2
1
0
-1
-2
-3
Baseline (C)
Change (C)
Baseline (T)
Change (T)

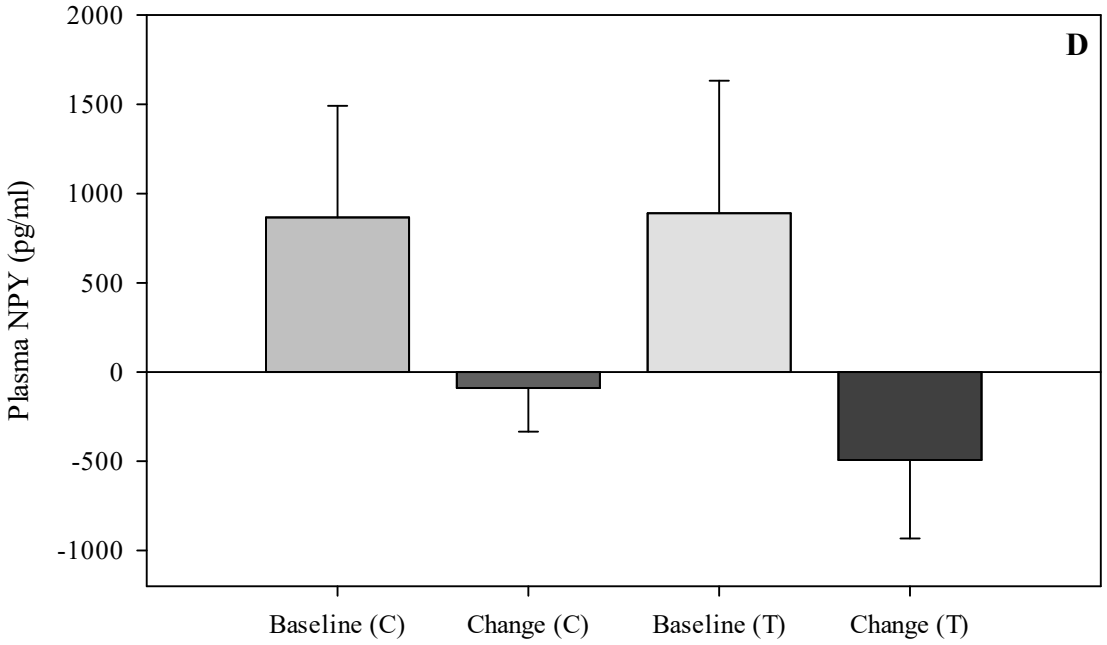


Figure 5.5: Change over 24 hours in blood glucose (A; baseline p=1.000 & change p=0.012), plasma ghrelin (B; baseline p=1.000 & change p=0.171), plasma LEAP2 (C; baseline p=1.000 & change p=0.203) and plasma NPY (D; baseline p=1.000 & change p=0.007) for acute food deprivation participants; 'C' = control and 'T' = treatment

There is limited previously published research regarding changes in these specific blood concentration variables following acute food deprivation. Similar to this research project, previously published research (Hojlund et al., 2001) as well as the other mentioned dissertation (Gonzalez, 2021) have shown decreased blood glucose concentration following a 24-hour bout of acute food deprivation. Additionally, previously published research has also shown increased plasma ghrelin concentration following a 24-hour bout of acute food deprivation (Espelund et al., 2005; Shiiya et al., 2002). No known

previously published research shows the response of plasma LEAP2 and NPY concentrations to acute food deprivation in humans.

Furthermore, these blood concentration changes were associated with the cardiovascular response to the 24-hour fast with the largest correlations for each cardiovascular variable being: glucose for systolic blood pressure (r=0.37), ghrelin for diastolic blood pressure (r=0.39), and glucose for heart rate (r=-0.44).

Additionally, considering only the blood biomarkers, the largest correlation occurred between glucose and ghrelin (r=-0.36), and other research has shown a relationship to exist between blood glucose and the other blood biomarkers tested in this research project. Previously published research has shown an antagonistic relationship between blood glucose, and ghrelin (Broglio et al., 2004) and NPY (Burdakov et al., 2005), and a protagonistic relationship between blood glucose and LEAP2 (Mani et al., 2019). However, the previously published research regarding the relationship between glucose and LEAP2 and NPY was with animals while this research project used humans. Compared to the existing previous research, this research project showed a similar relationship between glucose and ghrelin, but an opposite relationship between glucose, and LEAP2 and NPY.

Lastly, participants showed a decrease in body mass during fasting, and little to no change in physical activity level or stress level between the control and treatment condition measurements. Other previously published research has also shown a decrease in body mass after a 24-hour fast (Horne et al., 2013). And the previously mentioned

dissertation found no difference in physical activity level during a 24-hour fast (Gonzalez, 2021). No other known research has looked into changes in stress level occurring at the same time as a novel acute fast.

5.6 Summary

Part one of the two-part hypothesis of this dissertation research project was that a 24-hour bout of acute food deprivation would likely lead to an excitatory cardiovascular response in healthy humans, associated with hormonal changes. Specifically, it was expected that BP and HR would be increased after 24 hours of acute food deprivation, along with decreased blood glucose, increased plasma ghrelin, increased plasma NPY, and decreased plasma LEAP2. The results of this dissertation research project are in support of this hypothesis, except for the changes in plasma NPY.

Considering all of this, why are these changes found during and after a 24-hour period of acute food deprivation?

I speculate that during and after a 24-hour period of acute food deprivation, the primary driver of food-related cardiovascular response is food input.

The primary acute food deprivation cardiovascular findings of this dissertation research project were decreased blood pressure and heart rate during acute food deprivation and increased blood pressure and heart rate after acute food deprivation.

Regarding decreased BP and HR during acute food deprivation, as noted in chapter two, humans will typically eat every 4 to 7 hours while they are awake, and wait around 12

hours from their last to first meal, encompassing the overnight sleeping period (Ishihara et al., 1985). Thus, a typical human will eat two to three meals per day over a 12-hour period. Furthermore, research has shown there to be increased HR and slightly increased SBP immediately following food consumption (Ohara et al., 2015). And the in-depth review results (chapter 4) showed BP and HR to be higher at the 1-hour post-prandial mark compared to the 12-hour post-prandial mark. Additionally, this dissertation research project showed there to be decreased BP and HR throughout a 24-hour period of no food consumption, relative to a 24-hour period of normal food consumption, with the increased BP and HR during normal food consumption only existing during the awake period, when participants would have been eating.

Therefore, while awake during a period of normal food consumption the repeated cycles of eating could lead to repeated bouts of a food-related excitatory cardiovascular response, and therefore BP and HR would show increased values compared to a period of acute food deprivation. Thus, during acute food deprivation could be considered to be a more parasympathetic state. Furthermore, the changes in blood biomarkers (namely decreased plasma NPY) could have contributed to the decreased BP and HR. There is evidence that NPY facilitates increased cardiovascular activity both directly (Allen & Bloom, 1985) and indirectly (M. Wang et al., 2016). Thus, the overall decreased ambulatory BP and HR during acute food deprivation could also be related to the decreased plasma NPY levels seen at the end of the 24 hours of acute food deprivation.

While this research project showed lessened overall BP and HR during acute food deprivation, it also showed increased BP and HR after a 24-hour period of acute food

deprivation. Previous research has also shown increased DBP (Horne et al., 2013) and HR (Herbert et al., 2012) after 24 hours of acute food deprivation. Furthermore, the in-depth review results showed BP and HR to be at a higher value at the 24-hour post-prandial mark compared to the 12-hour post-prandial mark. The reason for this increased post-prandial value is perhaps connected to the timing of the measurements, and the relationship between the cardiovascular variables and certain blood biomarker variables.

For this research project, measurements were taken in the morning around the participants' normal breakfast time, and only approximately 30 minutes prior to participants being able to eat. At this point, participants had gone approximately 12 hours since eating in the normal food consumption condition (typical for them) and 24 hours since eating in the acute food deprivation condition (atypical for them). Previous research has shown that the primary nutrient uptake time occurs within the first 9 hours after eating (Fischer & Fadda, 2016; Sugita et al., 2019), which participants would have been well beyond after 24 hours of acute food deprivation. At the end of this 24-hour period of acute food deprivation blood glucose was decreased and plasma ghrelin was increased, beyond the normal food consumption condition. Blood glucose was likely decreased due to participants having not consumed food in the past 24 hours (no new glucose coming into the body), and this decrease in blood glucose (Broglio et al., 2004) coupled with ghrelin's natural circadian rhythm (peaking in the morning) (Espelund et al., 2005) along with participants anticipating their first meal in 24 hours is likely what led to the increased plasma ghrelin levels. And on top of this decrease in blood glucose

and increase in plasma ghrelin, there was also a decrease in plasma LEAP2, which would have allowed for ghrelin to potentially have a greater effect (Ge et al., 2018).

Therefore, participants' increased anticipation of eating in conjunction with / being driven by decreased blood glucose levels and increased plasma ghrelin levels, essentially occurring at the very end of the acute food deprivation period (and at the time of measurement), is likely what led to an increase in sympathetic activity which showed as increased BP and HR. This would be similar to the increase in ghrelin, BP and HR typically seen at meal times during normal food consumption, but exacerbated by the exaggerated (atypical) time between meals during acute food deprivation.

As noted in chapter two (section 2.4), the possible reason for the decrease in blood glucose and the increase in plasma ghrelin leading to the increased sympathetic response is an increase in sympathetic activity related to gluconeogenesis, and the downstream effects of the changes in glucose and ghrelin levels.

It should furthermore be noted that the only lifestyle (outside of fasting) or anthropometric change during acute food deprivation was a decrease in body mass, which was likely primarily due to the decrease of food within the GI tract during acute food deprivation. Participants in this research project did not indicate to have increased their water consumption during food deprivation, and the other dissertation (Gonzalez, 2021) from the same lab as this research project showed no change in hydration status after a 24-hour period of acute food deprivation. Furthermore, anecdotally, the blood samples for this research project seemed to indicate no change in hematocrit during acute food

deprivation. Therefore, any changes observed during acute food deprivation were not likely due to changes in lifestyle or anthropometrics.

All of these things together seem to point to the idea that acute food deprivation allows one to experience a longer period of time (relative to normal food consumption) with lessened sympathetic activity (more parasympathetic) during acute food deprivation, and also, seemingly, a boost of increased sympathetic activity at the end of the acute food deprivation period prior to food consumption.

Finally, with all of this in mind, it should also be mentioned that, yes, it appears that acute food deprivation causes an impact on cardiovascular physiology both during, and at the end of, the acute food deprivation period, but this impact seems to be somewhat mild. The changes in BP and HR seen are only a few millimeters of mercury and a few beats per minute. Present, seemingly, but small. Perhaps further study could help to clarify how (and how much) acute food deprivation impacts cardiovascular physiology.

6 Results & Discussion (Chronic Food Deprivation)

The previous chapter presented the acute food deprivation results along with a discussion of said results. This chapter will present the findings of the experimental protocol that focused on chronic food deprivation, along with a discussion on said findings.

6.1 Data Collection Overview

As noted previously, data collection for the entire research project took place from February of 2020 until May of 2021, with a variety of starts and stops along the way, due to COVID-19 pandemic restrictions. Pandemic contingency plan component three focused on chronic food deprivation, and more details are given below.

6.1.1 Pandemic Contingency Plan Component Three

Data collection for the pandemic contingency plan component three protocol began on March 26, 2021 and continued until May 20, 2021. The second pandemic-related suspension of in-person research activity ended prior to this data collection period, and no further suspensions of in-person research activity occurred during this data collection period. Thus, the in-person research activity suspensions did not affect data collection for the pandemic contingency plan component three protocol.

Approval was granted to enroll and complete data collection for up to 6 participants for this protocol. There were a total of 6 participants enrolled in the pandemic contingency

plan component three protocol, and all 6 participants (3 female / 3 male) completed all three testing sessions.

As a reminder, and reference, the following figure (figure 6.1) displays the experimental timeline for the pandemic contingency plan component three protocol.

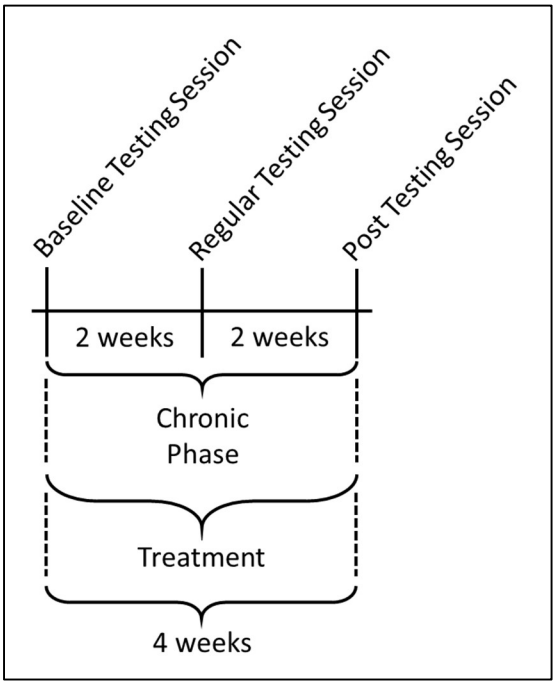


Figure 6.1: Experimental timeline of pandemic contingency plan component three protocol

For this protocol each participant completed three visits. The visits were spaced two weeks apart each. Visit 1 occurred at the beginning of the four-week chronic fasting regimen, visit 2 occurred in the middle, and visit 3 occurred at the end.

6.2 Chronic Food Deprivation Results

The chronic food deprivation results are organized below.

6.2.1 Demographics and Lifestyle Characteristics

The average age of the participants was 24 years with a standard deviation of 4.10 years. Regarding identity, participants gave 15 total answers; 7% of answers fit within the classification category and 93% fit within the personality category.

For the female participants, the average menstrual cycle phase was day 9.0±2.00 for visit 1, day 22.7±2.08 for visit 2, and day 9.3±2.52 for visit 3. The goal for the chronic food deprivation protocol was to have female participants come to their first lab visit within the first week (days 1-7) of their menstrual cycle, and so, hopefully, their third lab visit would also occur within the first week of the following menstrual cycle.

The average pre-visit fasting duration, for all participants, for all visits, was 11.1 hours (visit 1 = 11.4±1.43 hours; visit 2 = 11.5±1.53 hours; visit 3 = 10.4±1.62 hours). Furthermore, participants in this protocol were asked to complete a 24-hour breakfast-to-breakfast fast twice per week for four weeks. All participants except for two were able to complete all 8 fasts; those two participants completed 7 fasts each due to scheduling.

In regard to physical activity level, the average fitness level of participants was 2.3±0.52, and the average change in physical activity level was -0.3±0.82 from visit 1 to visit 2 and -0.3±0.52 from visit 2 to visit 3. And regarding stress level, the average total stress of

participants was 2.8±1.33, and the average change in stress level was -0.2±0.75 from visit 1 to visit 2 and 0.0±0.63 from visit 2 to visit 3.

6.2.2 Anthropometrics

The average height for all participants was 1.74±0.07 meters, and the remaining anthropometric variables are shown in the table (table 6.1) below.

Table 6.1: Anthropometric data for chronic food deprivation participants

Anthropometrics:

Chronic Food Deprivation

		Visit 1	Visit 2	Visit 3	p value
Mass (kg)	Average Sd	73.4 16.21	73.5 16.27	73.5 17.28	1.000
Fat Mass (%)	Average Sd	27.3 7.79	- -	26.7 7.22	0.095
Bone Mineral Content (g)	Average Sd	2666.3 425.57	- -	2683.2 433.79	0.365
Bone Mineral Density (g/cm^2)	Average Sd	1.22 0.13	- -	1.22 0.13	0.106

A one-way repeated measures ANOVA test with a Bonferroni post-hoc analysis was used to assess the mass data, and paired t-tests were used to assess the body composition data with resulting two-tailed p values being displayed.

6.2.3 Hemodynamics

The hemodynamic data are shown in the table below for seated rest (table 6.2).

Table 6.2: Seated rest hemodynamic data for chronic food deprivation participants

Hemodynamics (Resting Values):

Chronic Food Deprivation

		Visit 1	Visit 2	Visit 3	p value (1↔2)	p value (1↔3)
Systolic Blood Pressure (mmHg)	Average	117.5	108.7	107.3	0.011	0.004
	Sd	5.72	5.82	6.12		
Diastolic Blood Pressure (mmHg)	Average	71.7	65.2	66.7	0.071	0.202
	Sd	7.89	8.33	2.80		
Heart Rate (bpm)	Average	76.3	73.5	74.3	1.000	1.000
	Sd	9.18	11.04	8.76		

A one-way repeated measures ANOVA test with a Bonferroni post-hoc analysis was used to assess the hemodynamic data.

6.2.4 Blood Biomarkers

The blood biomarkers data are shown in the table below (table 6.3).

Table 6.3: Blood biomarkers data for chronic food deprivation participants

Blood Biomarkers (Resting Values): Chronic Food Deprivation						
		Visit 1	Visit 2	Visit 3	p value (1↔2)	p value (1↔3)
Blood Glucose (mg/dl)	Average	98.5	100.3	97.5	1.000	1.000
	Sd	7.79	5.35	11.12		
Plasma Ghrelin (pg/ml)	Average	89.4	91.8	159.7	1.000	0.372
	Sd	85.40	86.19	127.29		
Plasma LEAP2 (ng/ml)	Average	0.6	0.3	0.7	0.693	1.000
	Sd	0.90	0.64	0.80		
Plasma NPY (pg/ml)	Average	757.5	632.9	731.7	0.473	1.000
	Sd	586.82	448.06	527.5		

A one-way repeated measures ANOVA test with a Bonferroni post-hoc analysis was used to assess the blood biomarkers data.

6.2.5 Autonomic Function

The autonomic function data (during controlled breathing) are shown in the tables below (table 6.4A & table 6.4B).

Table 6.4A: Autonomic function data for chronic food deprivation participants

Autonomic Function:

Chronic Food Deprivation

		Pre	Post	p value
Mean Arterial Pressure (mmHg)	Average Sd	83.4 9.98	80.7 9.50	0.232
Systolic Arterial Pressure (mmHg)	Average Sd	108.9 12.25	105.2 7.01	0.168
Diastolic Arterial Pressure (mmHg)	Average Sd	64.1 9.01	62.5 10.78	0.359
Heart Rate (bpm)	Average Sd	76.2 10.68	71.9 8.94	0.087
R-to-R Interval (ms)	Average Sd	803.8 104.36	849.5 113.42	0.125
RRI-Low Frequency Power (ms^2)	Average Sd	606.2 691.93	581.3 831.03	0.471
RRI-High Frequency Power (ms^2)	Average Sd	1571.2 3062.85	1239.3 1679.03	0.304
Low Frequency / High Frequency Ratio (%)	Average Sd	0.94 0.96	0.76 0.78	0.293

Table 6.4B: Continuation of autonomic function data for chronic food deprivation participants

Autonomic Function:

Chronic Food Deprivation

		Pre	Post	p value
MAP-RRI Up-Up (ms/mmHg)	Average Sd	21.9 27.08	18.5 12.11	0.323
SAP-RRI Up-Up (ms/mmHg)	Average Sd	13.1 10.83	12.5 6.12	0.419
DAP-RRI Up-Up (ms/mmHg)	Average Sd	18.2 13.55	20.1 8.69	0.417
MAP-RRI Down-Down (ms/mmHg)	Average Sd	21.6 21.74	22.8 17.85	0.374
SAP-RRI Down-Down (ms/mmHg)	Average Sd	11.7 6.79	11.2 3.82	0.412
DAP-RRI Down-Down (ms/mmHg)	Average Sd	17.4 11.84	19.0 11.85	0.271
Burst Frequency (bursts/min)	Average Sd	6.7 2.47	10.1 7.74	0.263
Burst Incidence (bursts/100hb)	Average Sd	9.7 3.13	14.2 10.6	0.274

The “pre” time point refers to the first participant visit at the beginning of the 4-week chronic food deprivation period. The “post” time point refers to the third participant visit at the end of the 4-week chronic food deprivation period. All variables except for the MSNA variables were derived from all six participants. MSNA data was only able to be obtained on two of the participants; one female and one male. Paired t-tests were utilized to calculate the one-tailed p values derived from comparing the pre and post data.

6.2.6 Correlations

Correlation analysis was used to assess the relationship between changes in hemodynamics and autonomic function and changes in blood biomarkers throughout the chronic food deprivation period. The results of these correlation analyses are shown in the tables below (table 6.5A & table 6.5B).

Table 6.5A: Correlation analyses results for chronic food deprivation participants

Correlations:

Chronic Food Deprivation

	Change Over First Two Weeks		Change Over Second Two Weeks	
	r	r^2	r	r^2
SBP ↔ Glucose	-0.09	0.008	-0.03	0.001
SBP ↔ Ghrelin	0.15	0.02	-0.63	0.40
SBP ↔ LEAP2	-0.24	0.06	0.43	0.19
SBP ↔ NPY	0.68	0.47	-0.29	0.09
DBP ↔ Glucose	0.02	0.0002	-0.19	0.04
DBP ↔ Ghrelin	0.09	0.01	0.43	0.18
DBP ↔ LEAP2	-0.98	0.97	-0.49	0.24
DBP ↔ NPY	-0.29	0.08	-0.54	0.29
HR ↔ Glucose	-0.58	0.33	-0.23	0.05
HR ↔ Ghrelin	0.12	0.01	0.04	0.002
HR ↔ LEAP2	-0.43	0.18	-0.05	0.002
HR ↔ NPY	-0.04	0.002	-0.11	0.01

Table 6.5B: Continuation of correlation analyses results for chronic food deprivation participants

Correlations:

Chronic Food Deprivation

	Change From Pre to Post	
	r	r^2
RRI ↔ Glucose	0.60	0.36
RRI ↔ Ghrelin	-0.71	0.50
RRI ↔ LEAP2	-0.50	0.25
RRI ↔ NPY	-0.91	0.82
RRI-LF ↔ Glucose	0.44	0.19
RRI-LF ↔ Ghrelin	-0.86	0.73
RRI-LF ↔ LEAP2	-0.48	0.23
RRI-LF ↔ NPY	-0.99	0.99
RRI-HF ↔ Glucose	-0.02	0.0004
RRI-HF ↔ Ghrelin	-0.94	0.89
RRI-HF ↔ LEAP2	0.01	0.0002
RRI-HF ↔ NPY	-0.90	0.80

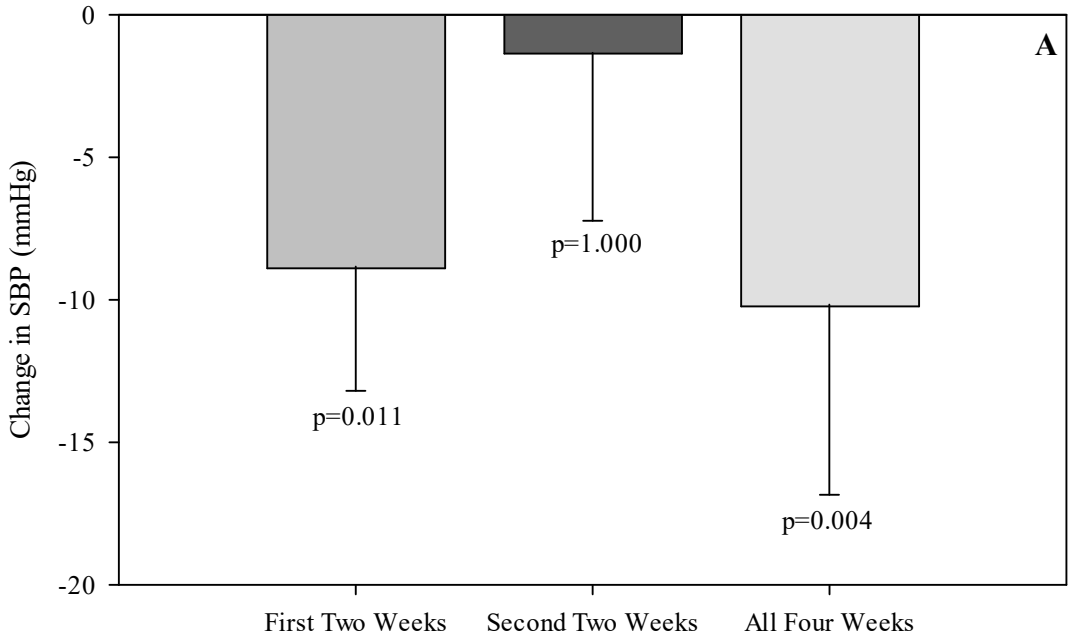
6.3 Chronic Food Deprivation Results Discussion

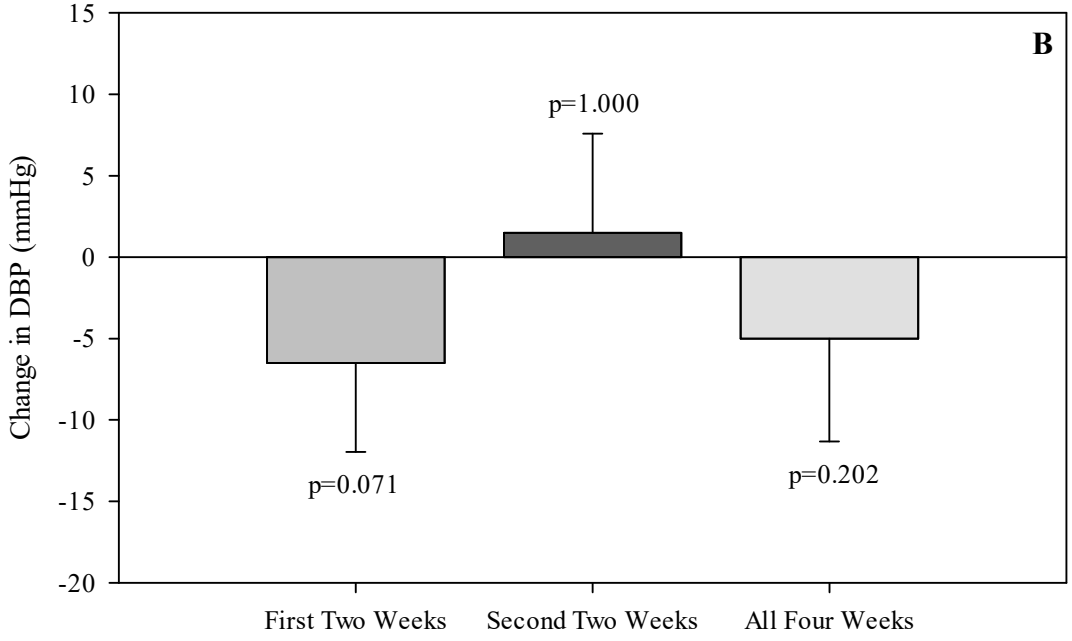
Regarding the chronic food deprivation protocol for this research project, the collected data showed decreased blood pressure, and little to no change in heart rate, during and

following the 4-week chronic food deprivation period. Additionally, BP and HR experienced the larger change during the first two weeks compared to the last two weeks.

The decrease in BP and HR also showed up in the autonomic function testing section, along with changes in heart rate variability. The autonomic function results showed decreased SAP, HR, RRI-HF, LF/HF ratio, and a small decrease to no change in DAP after four weeks of chronic food deprivation. And the autonomic function results showed increased RRI with a small increase to no change in RRI-LF.

These described hemodynamic and autonomic changes are illustrated in the figures below (figures 6.2, 6.3 & 6.4).





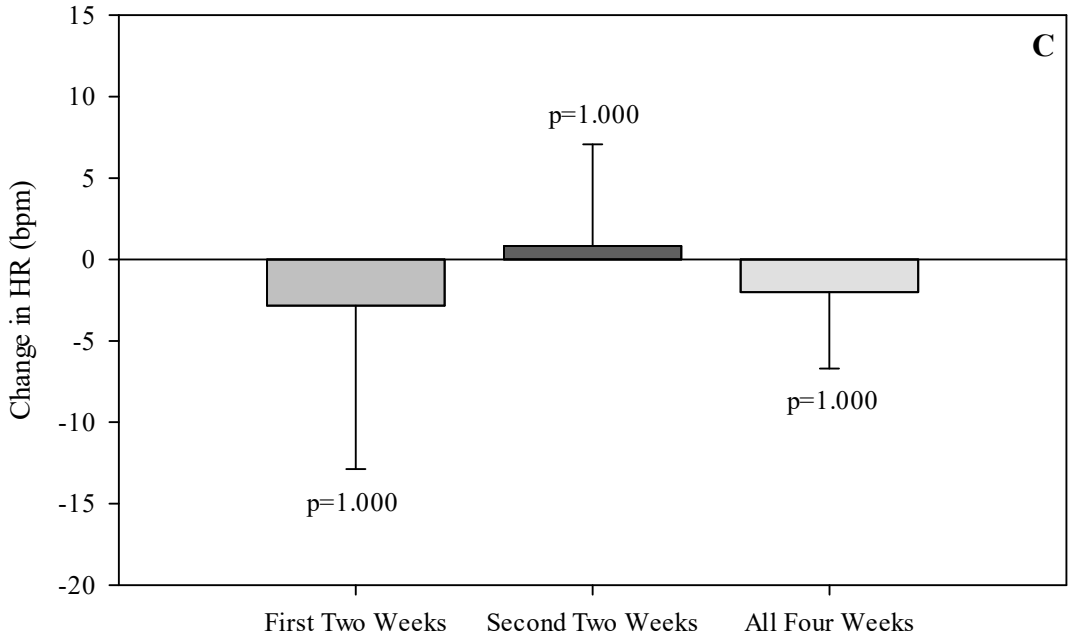


Figure 6.2: Change in systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C) throughout 4-week period for chronic food deprivation participants

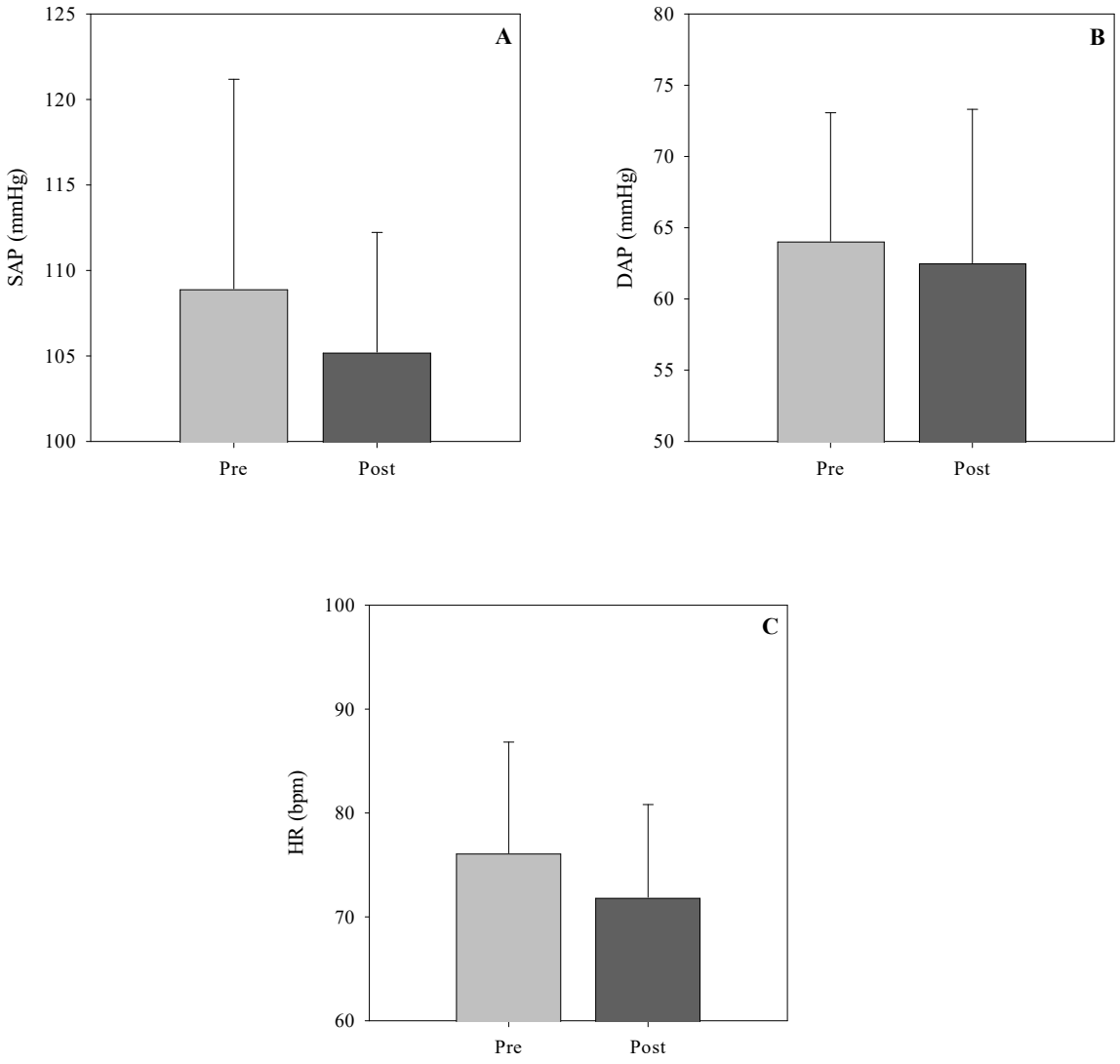


Figure 6.3: Change in systolic arterial pressure (A; p=0.168), diastolic arterial pressure (B; p=0.359) and heart rate (C; p=0.087) during the autonomic function test for chronic food deprivation participants

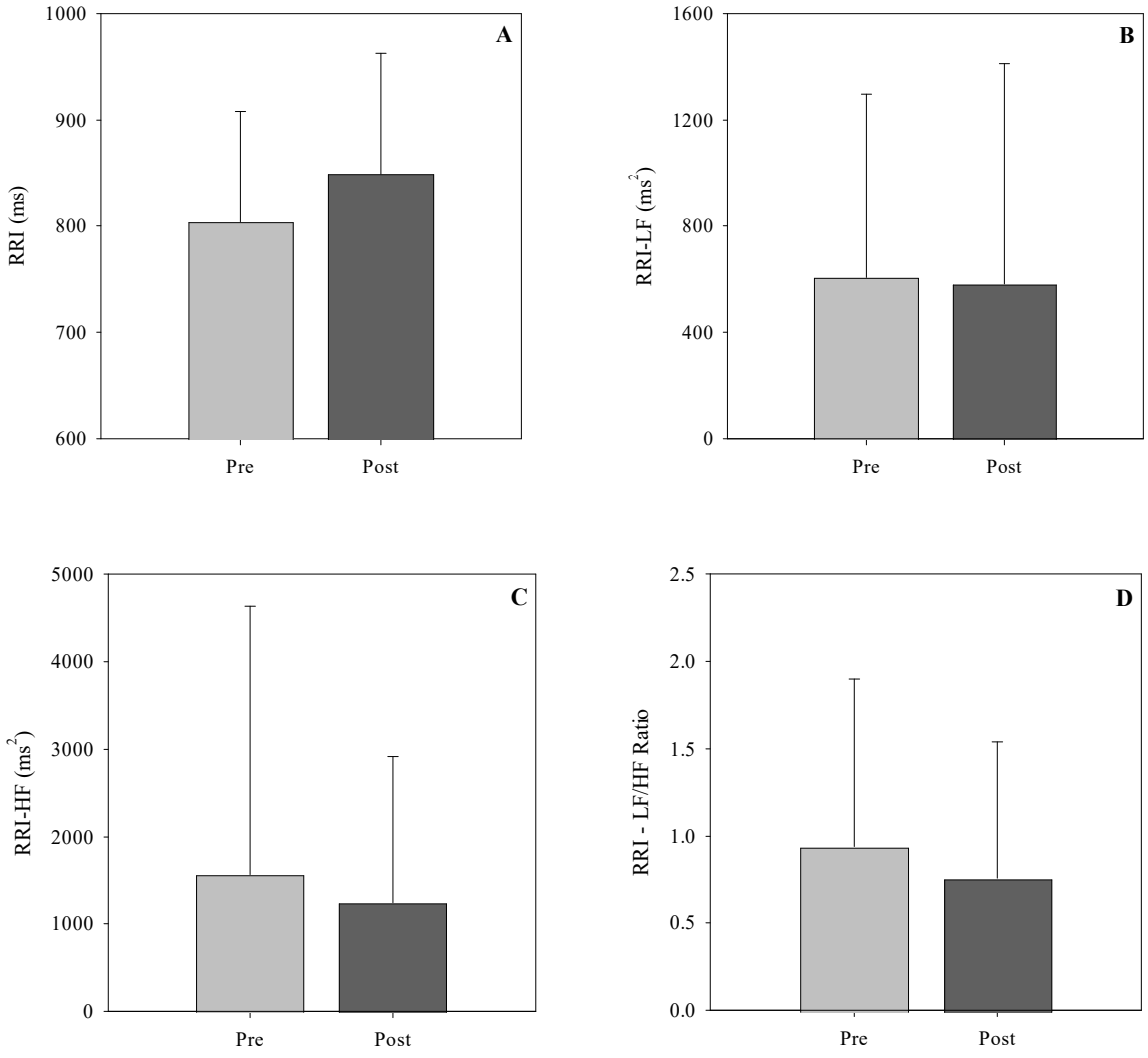


Figure 6.4: Change in RRI (A; p=0.125), RRI-LF (B; p=0.471), RRI-HF (C; p=0.304) and RRI-LF/HF Ratio (D; p=0.293) during the autonomic function test for chronic food deprivation participants

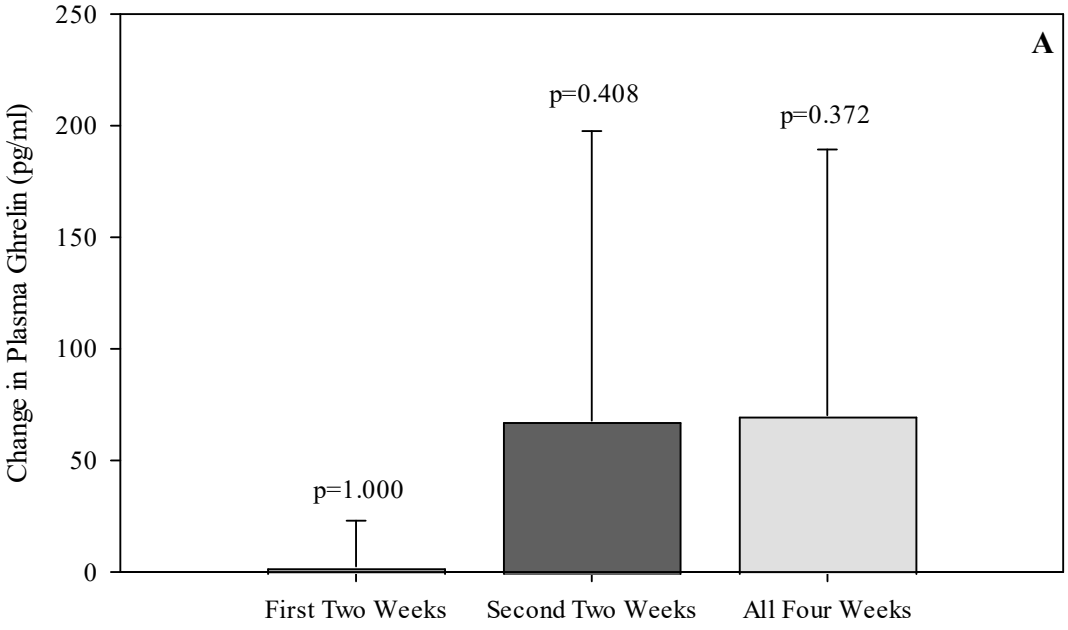
The chronic food deprivation hemodynamic results of this research project are generally in line with previously published data. Previously published data on chronic food deprivation (Ramadan) shows decreased SBP and DBP (Dewanti et al., 2006) and HR

(Samad et al., 2015), as well as increased DBP (Harder-Lauridsen et al., 2017) and HR (Cansel et al., 2014). When assessing these articles, as well as others in the in-depth review, what stands out is that the combined data supports the notion of a chronic food deprivation regimen (Ramadan) leading to a decrease in resting BP and HR. However, the in-depth review results show that this decrease is not necessarily linear nor do the greatest changes necessarily occur after two weeks. But, importantly, the previously published research almost exclusively utilizes Ramadan as the chronic food deprivation protocol, which is very different from the chronic food deprivation protocol used for this dissertation research project.

In regard to the autonomic function results, there is limited previously published research regarding chronic food deprivation and autonomic function. One previous study found heart rate variability to increase during Ramadan (Cansel et al., 2014). The results of this research project also showed heart rate variability to increase in the time domain, and with a shift toward low frequency (more associated with parasympathetic activity) in the frequency domain.

Along with these hemodynamic and autonomic changes, chronic food deprivation also led to changes in plasma ghrelin concentration, plasma LEAP2 concentration and plasma NPY concentration. Over the course of this chronic food deprivation regimen, plasma ghrelin concentration showed little to no change from baseline to week two, and then an increase from week two to the post measurement. Plasma LEAP2 and NPY concentrations were similar, showing a decrease from baseline to week two and then an increase from week two to post. Overall, after the 4-week chronic food deprivation

period, plasma ghrelin concentration was increased, while plasma LEAP2 and plasma NPY concentrations were at relatively baseline levels. These described changes in blood biomarkers are shown in the figure below (figure 6.5).



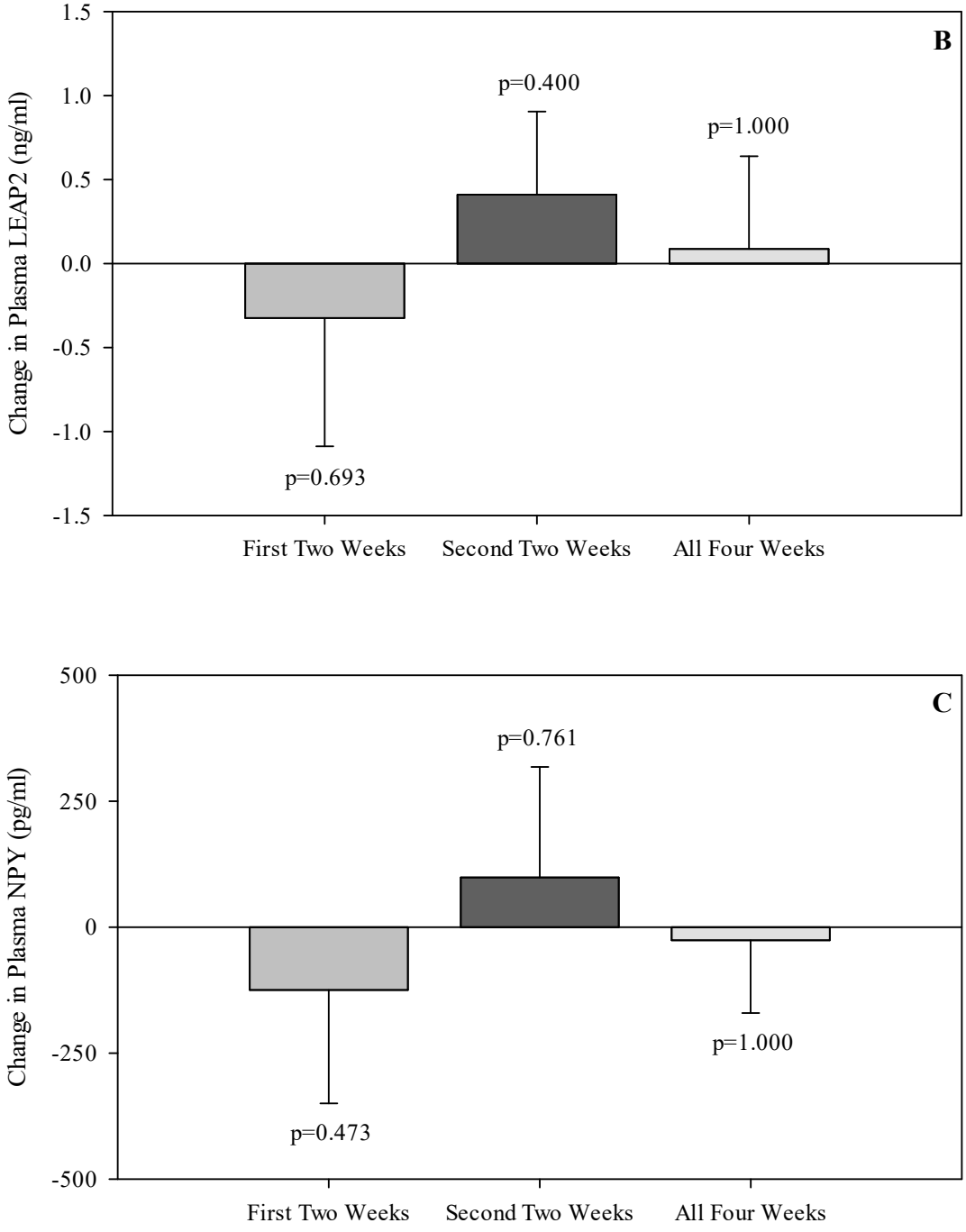


Figure 6.5: Change in plasma ghrelin (A), plasma LEAP2 (B) and plasma NPY (C) for chronic food deprivation participants

There is limited previously published research regarding chronic food deprivation and these plasma concentration variables. One study found ghrelin levels to increase after one month of two days per week of 25% caloric restriction (Harvie et al., 2011). No known previously published research shows how plasma LEAP2 and NPY concentrations change during or after chronic food deprivation.

These plasma concentration changes were also associated with the cardiovascular changes throughout the 4-week chronic food deprivation period with the largest correlations for each variable for the first two weeks being: NPY for SBP (r=0.68), LEAP2 for DBP (r=-0.98), and LEAP2 for HR (r=-0.43). The largest correlations for the second two weeks being: ghrelin for SBP (r=-0.63), NPY for DBP (r=-0.54), and NPY for HR (r=-0.11). And the largest correlations for the four weeks overall being: NPY for RRI (r=-0.91), NPY for RRI-LF (r=-0.99), and ghrelin for RRI-HF (r=-0.94).

Lastly, it should be noted that participants sustained the chronic food deprivation regimen with relatively no change in physical activity level, stress level, body mass, body composition, or blood glucose concentration. Furthermore, no participants indicated a change in sleep or dietary patterns throughout the chronic food deprivation period.

6.4 Summary

Part two of the two-part hypothesis of this dissertation research project was that experiencing a 24-hour bout of acute food deprivation twice per week for 4 weeks would lead to cardiovascular adaptations stemming from reduced basal sympathetic tone and

altered circulating hormone levels. Specifically, it was expected that BP and HR would be decreased after 4 weeks of chronic food deprivation, along with increased HRV, decreased plasma ghrelin, decreased plasma NPY, and increased plasma LEAP2. The results of this dissertation research project are in support of this hypothesis except for the changes in plasma ghrelin and plasma LEAP2.

Considering all of this, why are these changes found during and after a 4-week period of chronic food deprivation?

I speculate that the cardiovascular changes (adaptations?) that took place during chronic food deprivation were primarily driven by hormonal changes that led to increased basal parasympathetic tone.

The primary chronic food deprivation cardiovascular findings of this dissertation research project were decreased blood pressure at rest during and after chronic food deprivation, and decreased blood pressure and heart rate along with increased heart rate variability under controlled breathing conditions at the end of the chronic food deprivation period.

During chronic food deprivation, the largest decrease in BP occurred after the first two weeks and this decrease remained at the end of the entire four weeks, with little to no change during the second two weeks. SBP underwent a larger decrease than DBP. Coinciding with the decrease in BP during the first two weeks were also a decrease in plasma LEAP2 and plasma NPY, along with essentially no change in plasma ghrelin or blood glucose. Furthermore, this decrease in BP during the first two weeks was notably correlated to the decreases in LEAP2 and NPY, with the primary, positive correlation

being between SBP and NPY. During the second two weeks, BP underwent little to no change and there was still a notable correlation between BP and LEAP2 and NPY, but during these second two weeks there was also a large increase in the correlation between BP and plasma ghrelin. And it was during these second two weeks that plasma ghrelin concentration increased.

At the beginning and end of the 4-week chronic food deprivation period there was an autonomic function test that measured HRV, BTBBP and MSNA during controlled breathing (15 breaths per minute) at rest. The goal of the controlled breathing was to exclude respiratory sinus arrythmia (Yasuma & Hayano, 2004) while measuring cardiovascular function. During controlled breathing it was found that RRI (time domain) was increased (along with a decrease in BP and HR) at the end of the 4-week chronic food deprivation period, and the change in RRI was most correlated (in the time and frequency domain) with plasma ghrelin and plasma NPY. Along with the changes in RRI, there was also a decrease in the RRI LF/HF ratio, and some evidence of increased BRS, which is associated with increased vagal tone (Kollai, Jokkel, Bonyhay, Tomcsanyi, & Naszlady, 1994).

The above points seem to point toward the decreased BP and HR as a result of chronic food deprivation being indicative of increased basal parasympathetic tone that is associated with changes in hormonal concentrations.

As noted in chapter two, NPY can directly and indirectly lead to an increase in sympathetic activity. Therefore, it is possible that the initial decrease in NPY along with

essentially no change in ghrelin, followed by the end of chronic food deprivation slightly decreased NPY levels and slightly increased LEAP2 levels (decreasing the effect of ghrelin), precipitated a decreased sympathetic stimulus, and thus lessened sympathetic activity that remained despite plasma LEAP2 and plasma NPY levels returning to baseline at the end of the 4 weeks of chronic food deprivation.

Although plasma LEAP2 and plasma NPY returned to baseline levels by the end of the chronic food deprivation period, plasma ghrelin was found to be increased. Perhaps the more long-term (post 2 weeks) adaption to chronic food deprivation that facilitates decreased BP is this increase in plasma ghrelin. There is previously published research that discusses the inverse correlation between circulating ghrelin concentrations and BP (Mao, Tokudome, & Kishimoto, 2016). Additionally, the decrease in plasma LEAP2 after 2 weeks could have allowed for ghrelin to have more of an effect and contribute to the reduction in BP seen.

Furthermore, it should be noted that since participants were able to maintain the 4-week chronic food deprivation regimen with essentially no change in lifestyle or anthropometrics, so it is likely that the cardiovascular changes were not influenced by lifestyle (outside of fasting) or anthropometrics.

Finally, similar to acute food deprivation, yes, it appears that chronic food deprivation causes an impact on cardiovascular physiology, but this impact seems to be somewhat mild. The changes in BP seen are 10 mmHg or less, and the changes in HR seen are just a few beats per minute. Mild, yes, but still meaningful for someone who's health could

benefit from a reduction in BP. Again, perhaps further study could help to clarify how (and how much) chronic food deprivation impacts cardiovascular physiology.

7 Conclusion

The previous three chapters detailed the results of this dissertation research project along with a discussion on said results. This final chapter serves as a conclusion for this dissertation research project.

7.1 Novel Aspects of this Research Project

There were several novel aspects of this research project, and the primary novel aspects are noted below.

Regarding the in-depth review, there was only one previously published systematic review and meta-analysis article that was found that covered fasting and blood pressure (Kord-Varkaneh et al., 2020), but this article focused primarily on research studies that utilized energy restricting diets as opposed to complete food deprivation and only utilized chronic protocols. Therefore, this research project includes the only known in-depth review that covers how blood pressure and heart rate are affected by both acute and chronic food deprivation in healthy, adult human participants.

Regarding acute food deprivation, the novel aspects of this research project were: 1) the 24-hour ambulatory assessment of blood pressure and heart rate during a 24-hour breakfast-to-breakfast water-only fast in healthy, adult humans, and 2) the assessment of plasma LEAP2 and plasma NPY concentrations after a 24-hour water-only fast in healthy, adult humans.

Regarding chronic food deprivation, the novel aspects of this research project were the assessment of hemodynamics, plasma biomarkers, and autonomic function both during and following a chronic food deprivation regimen consisting of two 24-hour water-only fasts per week for a period of four weeks.

7.2 Research Project Limitations

There were some limitations to this research project, and the main limitations are noted below.

One, the experimental protocols were considerably affected by restrictions related to the COVID-19 pandemic (ex. multiple protocols crafted, multiple participant pools, 4-week chronic protocol instead of 6 weeks).

Two, the number of participants was less than desired, mainly due to the restrictions related to the COVID-19 pandemic, as well as difficulty in obtaining certain measurements (ex. MSNA) on all participants.

Three, outside of the ambulatory measurements, the rest of the measurements for the acute food deprivation protocol were only taken before and after, and not during, acute food deprivation.

Four, the chronic food deprivation measurements were taken only at baseline conditions (i.e. in the morning after an overnight fast), and not after a 24-hour fast, therefore each 24-hour fast throughout the chronic food deprivation period was not assessed individually.

Five, the autonomic function data was only collected before and after the 4-week chronic food deprivation period, and not during (ex. at the 2-week mid-point mark) due to some measurements (ex. MSNA) not being able to be done at a less than 4-week separation.

Six, there were some complexities with some of the blood biomarkers data. In some instances (ex. LEAP2 and NPY) there was limited prior data to compare. And in other instances, the biomarker value mean was less than the spread (standard deviation). This could be related to a variety of factors, such as the measurement kit and measurement protocol utilized.

Seven, there was a wide range in the 'p' and 'r' values generated from the statistical analyses. A larger p value or a smaller r value means that the statistical probability of significant results is reduced. This was likely related to the reduced number of research participants, any instances of a large standard deviation and/or any instances of a lower statistical power value.

Eight, the timing of the laboratory visits was primarily based upon the availability of participants and although all visits occurred in the morning prior to participants' first meal, all visits were not able to occur at the exact same time of day.

7.3 Thoughts on Future Research

Regarding research on the impact of food deprivation on cardiovascular physiology going forward, I think that it would be informative/helpful to explore the following avenues.

One, look into the effect of various acute fasting durations within a 24-hour period (ex. 0hr, 2hr, 4hr, 6hr, 8hr, 10hr, 12hr, 14hr, 16hr, 18hr, 20hr, 22hr, 24hr).

Two, perform a 24-hour assessment of more variables beyond blood pressure and heart rate (ex. EKG, LEAP2, NPY, etc.), and with each participant following the same timing schedule.

Three, better define what constitutes a baseline acute fed state.

Four, test more, different chronic food deprivation protocols, and for longer periods of time (ex. fasting more or less than twice per week, and greater than four weeks).

Five, look into the effect of coupling food deprivation (both acute and chronic) with other modalities (ex. exercise, meditation, etc.).

7.4 Final Summary

In grand summary, it appears that the impact that food deprivation has on cardiovascular physiology is a mild but positive one, both acutely and chronically. Although this impact appears positive, it is also complex, nuanced, and there is still much to uncover. Acutely, it appears that food deprivation allows for a longer, more sustained period of lessened sympathetic activity. Chronically, it appears that food deprivation allows for this lessened sympathetic activity during acute food deprivation to be translated to a baseline state of lessened sympathetic activity.

Not only is food deprivation, as prescribed in this research project, doable, but the overwhelming body of epidemiological research also suggests that regular food deprivation is a healthy lifestyle choice, and the results of this research project seem to support that.

Sometimes, to give up what we want, is better than holding onto what we think we need.

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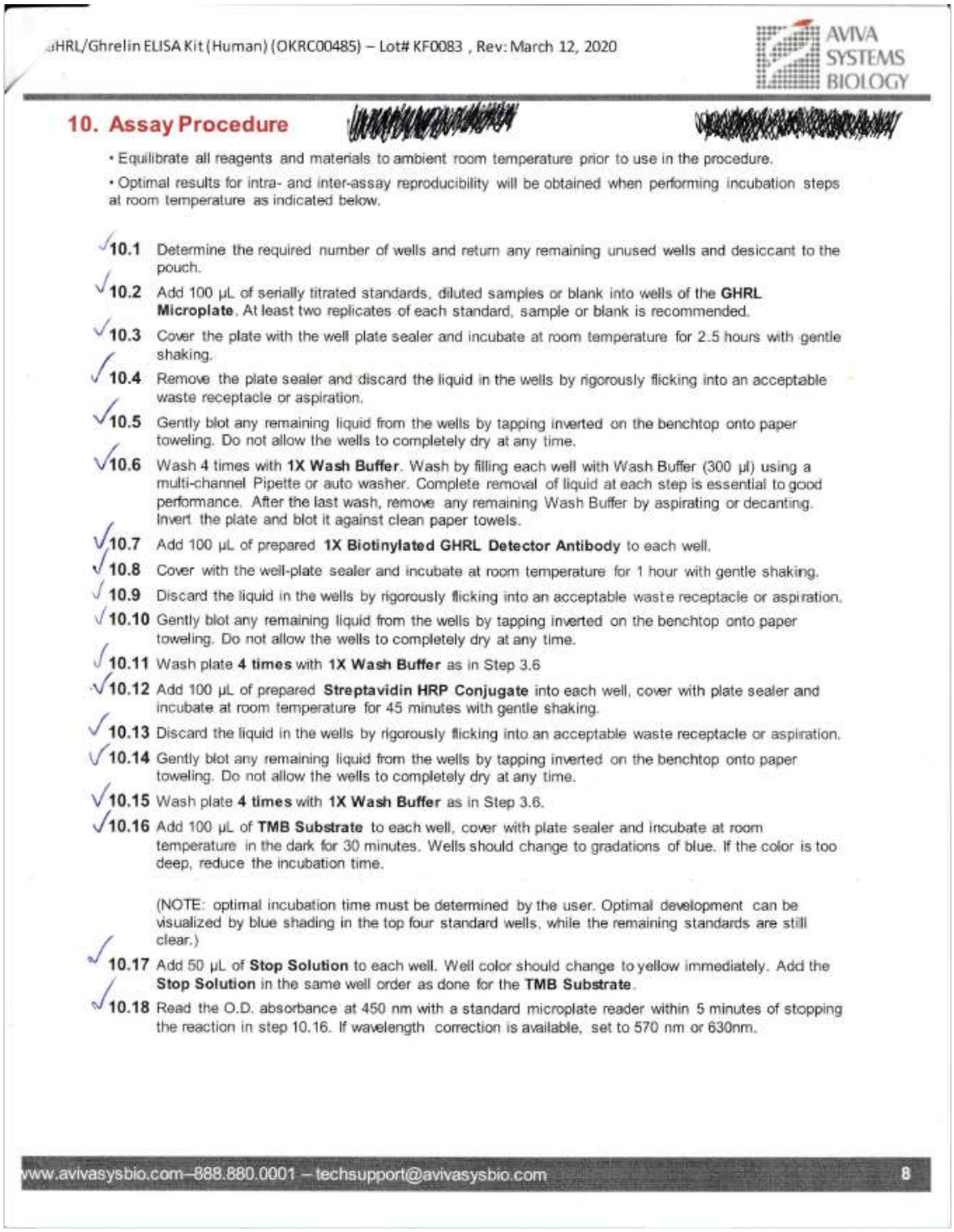
A Appendix A (ELISA Kit Instructions)

A.1 Ghrelin ELISA Kit Instructions



AVIVA SYSTEMS BIOLOGY

10. Assay Procedure

- Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
- Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at room temperature as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **GHRL Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate sealer and incubate at room temperature for 2.5 hours with gentle shaking.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Wash 4 times with **1X Wash Buffer**. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

10.7 Add 100 µL of prepared **1X Biotinylated GHRL Detector Antibody** to each well.

10.8 Cover with the well-plate sealer and incubate at room temperature for 1 hour with gentle shaking.

10.9 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.10 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.11 Wash plate **4 times** with **1X Wash Buffer** as in Step 3.6

10.12 Add 100 µL of prepared **Streptavidin HRP Conjugate** into each well, cover with plate sealer and incubate at room temperature for 45 minutes with gentle shaking.

10.13 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.14 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.15 Wash plate **4 times** with **1X Wash Buffer** as in Step 3.6.

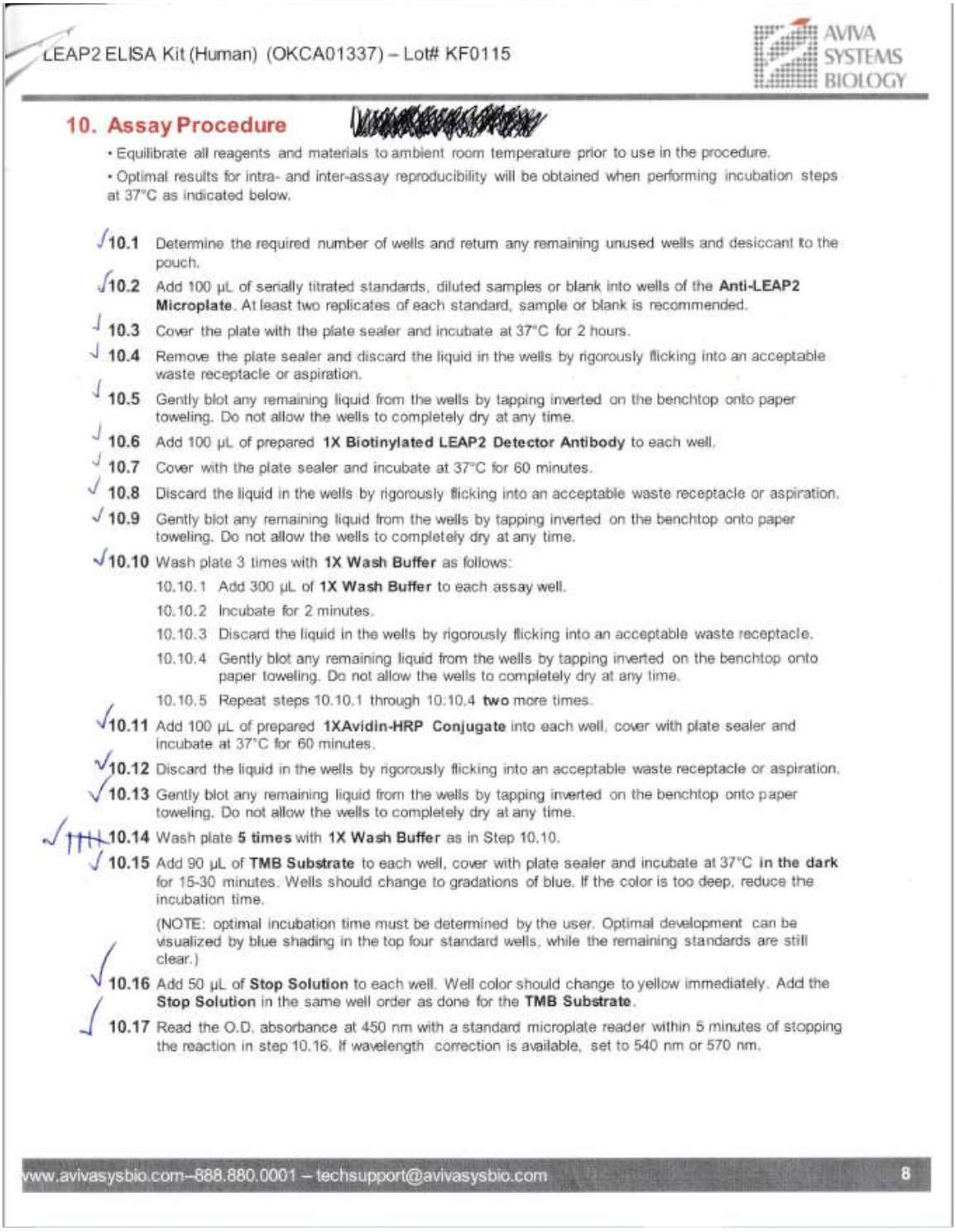
10.16 Add 100 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at room temperature in the dark for 30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.17 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.18 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 570 nm or 630nm.

A.2 LEAP2 ELISA Kit Instructions



LEAP2 ELISA Kit (Human) (OKCA01337) – Lot# KF0115

AVIVA SYSTEMS BIOLOGY

10. Assay Procedure

- Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
- Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-LEAP2 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the plate sealer and incubate at 37°C for 2 hours.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated LEAP2 Detector Antibody** to each well.

10.7 Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 2 minutes.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1XAvidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 60 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.17 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

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A.3 NPY ELISA Kit Instructions

10. Assay Procedure

- Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
- Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **NPY Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate sealer and incubate at 37°C for 2 hours.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated NPY Detector Antibody** to each well.

10.7 Cover with the well-plate sealer and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

- 10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.
- 10.10.2 Incubate for 1 minute.
- 10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- 10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1X Avidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 60 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.17 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

B Appendix B (Raw Data)

B.1 Acute Food Deprivation (Primary Outcome Variables)

ID	Visit	Sex	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Glucose (mg/dl)	Ghrelin (pg/ml)	LEAP2 (ng/ml)	NPY (pg/ml)
1	1	F	116	65	73	95	48.20	-0.44	1449.62
	2		97	57	76	89	55.27	-0.57	935.56
	3		95	51	63	91	31.57	-0.41	1314.10
	4		96	54	64	75	32.98	-0.14	399.76
2	1	M	111	51	55	86	5.39	-0.42	-285.27
	2		117	56	56	74	12.11	-0.31	-231.52
	3		114	53	48	95	22.37	-0.25	-262.40
	4		113	57	52	79	324.88	-0.42	-311.01
3	1	M	127	76	75	125	375.83	4.44	858.94
	2		122	74	73	118	353.18	3.04	706.83
	3		125	69	67	117	332.66	3.09	735.99
	4		119	73	68	117	239.26	2.39	355.16
4	1	M	105	60	50	91	129.57	-0.15	1009.32
	2		116	66	47	87	77.21	0.19	697.11
	3		112	66	56	93	38.65	0.41	434.07
	4		121	63	58	82	74.73	0.12	364.88
5	1	F	110	65	66	94	311.43	1.57	1691.50
	2		98	64	72	99	339.38	-0.17	1471.35
	3		100	65	73	97	210.95	0.08	1385.58
	4		101	64	72	87	187.25	0.32	1069.37
6	1	F	113	68	75	100	397.76	-0.09	1604.59
	2		107	68	69	115	323.46	-0.12	1602.87
	3		107	69	78	97	366.63	0.23	1928.24
	4		104	72	76	87	811.72	0.08	1730.96
7	1	F	96	62	78	81	138.42	-0.07	1449.62
	2		92	60	76	96	97.73	-0.20	1903.08
	3		94	66	88	89	89.95	-0.07	2056.32
	4		95	61	88	87	46.43	-0.49	907.54

8	1	M	110	68	66	80	482.68	-0.54	290.55
	2		107	66	66	84	484.09	-0.76	244.80
	3		125	77	69	85	343.98	-0.40	248.23
	4		122	75	76	77	176.63	-0.67	-206.94
9	1	M	112	80	76	106	186.89	0.29	702.26
	2		103	73	85	95	161.42	0.24	752.01
	3		114	74	72	109	130.99	0.25	854.93
	4		116	73	88	99	123.91	0.04	225.93
10	1	M	123	79	63	100	110.11	-0.03	-160.62
	2		122	67	64	96	134.17	-0.30	-289.85
	3		114	69	68	94	124.97	-0.27	-166.34
	4		116	70	72	82	186.18	3.78	54.39
11	1	M	130	88	94	117	27.68	0.45	1336.98
	2		131	87	101	101	8.57	0.00	1524.53
	3		131	89	91	99			
	4		133	86	87	98	-111.02	0.11	887.53
12	1	M	123	81	90	91	59.52	0.16	557.59
	2		122	79	71	95	69.43	0.06	567.31
	3		119	75	78	88	110.11	2.28	983.02
	4		131	78	76	96	45.72	-0.08	165.32
13	1	F	115	68	71	80	150.80	-0.40	580.46
	2		107	63	79	89	73.32	-0.42	634.21
	3		110	60	79	82	62.00	-0.12	479.25
	4		107	62	82	66	40.77	-0.18	64.68
14	1	F	83	54	87	95	11.76	5.31	1041.35
	2		94	59	95	94	30.86	0.22	640.50
	3		89	59	77	93	45.01	3.76	1585.72
	4		88	59	90	78	58.46	-0.13	344.30

ID	Cond	Sex	Average SBP (mmHg)			Average DBP (mmHg)			Average HR (bpm)		
			All	Awk	Asl	All	Awk	Asl	All	Awk	Asl
1	Cont	F	115.33	117.04	110.90	63.06	66.62	53.80	68.56	70.50	63.50
2	Cont	F	110.40	118.54	100.33	63.15	72.88	51.10	71.34	71.65	70.95
3	Cont	M	121.02	126.52	111.13	67.12	74.15	54.47	59.52	63.30	52.73
4	Cont	M	125.40	134.54	110.56	71.36	77.81	60.88	55.54	57.32	52.75
5	Cont	M	119.95	130.09	103.29	68.43	77.35	53.79	77.73	82.39	70.07
6	Cont	F	113.46	117.13	105.87	64.61	70.29	52.87	65.02	65.26	64.53
7	Cont	F	116.81	121.35	105.08	72.49	77.03	60.75	66.98	74.00	48.83
8	Cont	M	124.96	132.06	102.36	73.72	78.83	57.45	72.33	77.03	57.36
9	Cont	M	124.98	126.36	121.71	75.72	78.09	70.14	74.72	77.19	69.07
10	Cont	M	116.00	118.76	109.50	65.51	69.12	57.00	54.91	58.06	47.50
11	Cont	M	139.36	144.38	129.67	79.55	83.48	71.93	80.02	83.45	73.40
13	Cont	F	113.79	118.68	104.67	64.34	71.31	50.87	66.75	72.17	56.27
14	Cont	F	113.93	117.30	105.38	65.24	69.12	55.38	74.83	82.09	56.38
1	Treat	F	115.33	120.76	106.06	63.38	69.29	53.65	66.22	68.45	62.41
2	Treat	F	109.93	115.54	102.65	57.50	64.19	48.80	69.00	75.15	61.00
3	Treat	M	125.47	132.76	115.33	69.33	76.96	58.72	50.77	51.32	50.00
4	Treat	M	125.55	128.03	120.73	70.57	74.28	63.40	60.84	61.34	59.87
5	Treat	M	115.77	119.24	102.67	65.42	68.09	55.33	68.51	70.97	59.22
6	Treat	F	115.34	120.77	105.76	70.06	77.80	56.41	60.53	62.10	57.76
7	Treat	F	107.45	114.31	94.71	65.60	72.19	53.36	65.15	71.27	53.79
8	Treat	M	115.57	122.50	105.56	65.05	70.65	56.94	68.16	75.42	57.67
9	Treat	M	126.73	128.59	120.40	81.28	82.58	77.00	74.00	74.62	71.90
10	Treat	M	119.70	121.68	115.60	64.72	68.06	57.80	61.54	63.48	57.53
11	Treat	M	138.50	146.23	124.00	78.46	84.37	67.38	78.80	83.00	70.94
13	Treat	F	111.50	113.76	106.46	65.83	69.83	56.92	70.43	72.86	65.00
14	Treat	F	108.63	111.21	102.08	62.07	67.15	49.15	68.02	72.30	57.15

B.2 Chronic Food Deprivation (Primary Outcome Variables)

ID	Visit	Sex	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Glucose (mg/dl)	Ghrelin (pg/ml)	LEAP2 (ng/ml)	NPY (pg/ml)
1	1	M	116	66	60	104	51.03	1.80	910.97
	2		111	70	66	103	49.61	-0.03	688.53
	3		110	68	61	108	19.89	0.91	663.95
2	1	F	113	69	80	86	84.64	-0.28	541.58
	2		103	62	83	90	112.59	-0.34	562.73
	3		106	66	84	86	91.36	-0.18	526.14
3	1	M	124	67	72	106	12.11	0.02	-8.51
	2		119	55	68	100	-9.47	0.38	-37.68
	3		112	63	70	106	309.31	-0.06	196.77
4	1	F	110	73	79	98	99.85	-0.13	730.28
	2		104	64	77	105	125.33	-0.22	886.38
	3		99	69	77	90	193.26	0.37	731.99
5	1	M	118	68	81	93	37.58	0.69	594.76
	2		107	61	59	103	37.58	0.61	407.77
	3		115	64	71		43.60	1.36	530.71
6	1	F	124	87	86	104	251.29	1.59	1776.13
	2		108	79	88	101	235.36	1.37	1289.52
	3		102	70	83		300.46	1.83	1740.68

ID	Sex	Visit	EKG					BtB-BP		
			HR (bpm)	RRI (ms)	RRI-LF (ms^2)	RRI-HF (ms^2)	LF/HF	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)
1	M	1	69.7	864	980	350	2.80	102	70.9	85
		2	57.5	1050	2223	1233	1.80	95.4	52.1	70.7
2	F	1	73.3	821	260	517	0.50	111.2	63.9	85
		2	74.1	811	96	228	0.42	105.7	62.9	80.7
3	M	1	66.4	921	1851	7818	0.24	109.7	56.1	75.2
		2	69.4	873	662	4566	0.14	106.5	55.8	75.2
4	F	1	70.8	849	353	315	1.12	88.9	52.4	69.1
		2	71.8	837	108	520	0.21	101.3	61.4	78.8
5	M	1	81.3	739	119	301	0.40	118.8	64.4	88.7
		2	73.2	822	211	780	0.27	105.7	60	80.2
6	F	1	95.4	629	74	126	0.59	123	76.7	97.3
		2	85.3	704	188	109	1.72	116.7	83	98.5

B.3 In-Depth Review

ID	Point (post-prandial)	SBP (mmHg)	DBP (mmHg)	HR (bpm)
1	1hr			66.5
	12hr			62.2
2	12hr	120.2	76.1	80.8
	48hr	115.6	73.9	76.2
3	1hr	117	71	67
	12hr	116	71.5	62.5
4	1hr			74
	18hr			73.4
5	24hr	110.9	68.9	
6	1hr			66.7
	24hr			71.3
7	12hr	104.5	55.5	60.8
	72hr	107.7	56.2	69.4
8	12hr	99.5	60.7	58.8

ID	Point (Ramadan)	SBP (mmHg)	DBP (mmHg)	HR (bpm)
1	Before	125.4	71.3	
	After	124.2	73.1	
2	Before	124.4	78.8	82.4
	Week 1	118.4	77	
	Week 2	120.5	74.3	
	Week 3	118.8	74.2	
3	Week 2			78
	After			80.1
4	Before	134	88	
	Week 3	124	77	

C Appendix C (Statistical Analysis Print Outs)

C.1 Acute Food Deprivation (Primary Outcome Variables)

One Way Repeated Measures Analysis of Variance

Dependent Variable: HR (bpm)

Normality Test (Shapiro-Wilk): Passed (P = 0.295)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.812)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	0	72.786	12.473	3.333
2.000	14	0	73.571	14.081	3.763
3.000	14	0	71.929	11.526	3.080
4.000	14	0	74.929	11.652	3.114

Source of Variation	DF	SS	MS	F	P
Between Subjects	13	6847.589	526.738		
Between Treatments	3	68.196	22.732	0.713	0.550
Residual	39	1244.054	31.899		
Total	55	8159.839	148.361		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	Diff of Means	t	P	P<0.050
4.000 vs. 3.000	3.000	1.405	1.000	No
4.000 vs. 1.000	2.143	1.004	1.000	Do Not Test
4.000 vs. 2.000	1.357	0.636	1.000	Do Not Test
2.000 vs. 3.000	1.643	0.770	1.000	Do Not Test
2.000 vs. 1.000	0.786	0.368	1.000	Do Not Test
1.000 vs. 3.000	0.857	0.402	1.000	Do Not Test

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.391)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1 to 2 (HR)	14	0	0.786	7.392	1.976
3 to 4 (HR)	14	0	3.000	5.684	1.519
Difference	14	0	-2.214	6.874	1.837

t = -1.205 with 13 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -6.183 to 1.755

Two-tailed P-value = 0.250

One-tailed P-value = 0.125

Paired t-test:

Dependent Variable: Overall HR (bpm)

Normality Test (Shapiro-Wilk): Passed (P = 0.544)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	68.462	7.923	2.197
2.000	13	0	66.385	6.862	1.903
Difference	13	0	2.077	4.907	1.361

t = 1.526 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.888 to 5.042

Two-tailed P-value = 0.153

One-tailed P-value = 0.0764

Paired t-test:

Dependent Variable: Awake HR (bpm)

Normality Test (Shapiro-Wilk): Passed (P = 0.193)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	71.769	8.786	2.437
2.000	13	0	69.231	8.217	2.279
Difference	13	0	2.538	5.532	1.534

t = 1.654 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.804 to 5.881

Two-tailed P-value = 0.124

One-tailed P-value = 0.0620

Paired t-test:

Dependent Variable: Sleep HR (bpm)

Normality Test (Shapiro-Wilk): Passed (P = 0.734)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	60.308	8.731	2.422
2.000	13	0	60.385	6.131	1.700
Difference	13	0	-0.0769	6.788	1.883

t = -0.0409 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -4.179 to 4.025

Two-tailed P-value = 0.968

One-tailed P-value = 0.484

One Way Repeated Measures Analysis of Variance

Dependent Variable: SBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.766)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.224)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	0	112.429	12.308	3.290
2.000	14	0	109.643	12.169	3.252
3.000	14	0	110.643	12.555	3.356
4.000	14	0	111.571	13.569	3.626

Source of Variation	DF	SS	MS	F	P
Between Subjects	13	7188.214	552.940		
Between Treatments	3	60.429	20.143	0.684	0.567
Residual	39	1149.071	29.463		
Total	55	8397.714	152.686		

Power of performed test with alpha = 0.050: 0.050

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 2.000	2.786	1.358	1.000	No
1.000 vs. 3.000	1.786	0.870	1.000	Do Not Test
1.000 vs. 4.000	0.857	0.418	1.000	Do Not Test
4.000 vs. 2.000	1.929	0.940	1.000	Do Not Test
4.000 vs. 3.000	0.929	0.453	1.000	Do Not Test
3.000 vs. 2.000	1.000	0.487	1.000	Do Not Test

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.959)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1 to 2 (SBP)	14	0	-2.786	8.359	2.234
3 to 4 (SBP)	14	0	0.929	4.747	1.269
Difference	14	0	-3.714	8.471	2.264

t = -1.641 with 13 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -8.605 to 1.177

Two-tailed P-value = 0.125

One-tailed P-value = 0.0624

Paired t-test:

Dependent Variable: Overall SBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.147)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	119.538	7.666	2.126
2.000	13	0	118.231	8.974	2.489
Difference	13	0	1.308	4.516	1.253

t = 1.044 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.422 to 4.037

Two-tailed P-value = 0.317

One-tailed P-value = 0.159

Paired t-test:

Dependent Variable: Awake SBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.133)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	124.846	8.444	2.342
2.000	13	0	122.846	9.441	2.619
Difference	13	0	2.000	5.831	1.617

t = 1.237 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.524 to 5.524

Two-tailed P-value = 0.240

One-tailed P-value = 0.120

Paired t-test:

Dependent Variable: Sleep SBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 1.000)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	109.308	8.390	2.327
2.000	13	0	109.462	8.781	2.435
Difference	13	0	-0.154	5.414	1.501

t = -0.102 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3.425 to 3.118

Two-tailed P-value = 0.920

One-tailed P-value = 0.460

One Way Repeated Measures Analysis of Variance

Dependent Variable: DBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.473)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	0	68.929	10.702	2.860
2.000	14	0	67.071	8.731	2.333
3.000	14	0	67.286	9.918	2.651
4.000	14	0	67.643	9.052	2.419

Source of Variation	DF	SS	MS	F	P
Between Subjects	13	4357.232	335.172		
Between Treatments	3	29.054	9.685	0.809	0.496
Residual	39	466.696	11.967		
Total	55	4852.982	88.236		

Power of performed test with alpha = 0.050: 0.050

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 2.000	1.857	1.420	0.981	No
1.000 vs. 3.000	1.643	1.257	1.000	Do Not Test
1.000 vs. 4.000	1.286	0.983	1.000	Do Not Test
4.000 vs. 2.000	0.571	0.437	1.000	Do Not Test
4.000 vs. 3.000	0.357	0.273	1.000	Do Not Test
3.000 vs. 2.000	0.214	0.164	1.000	Do Not Test

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.980)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1 to 2 (DBP)	14	0	-1.857	5.097	1.362
3 to 4 (DBP)	14	0	0.357	2.925	0.782
Difference	14	0	-2.214	6.179	1.651

t = -1.341 with 13 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -5.782 to 1.353

Two-tailed P-value = 0.203

One-tailed P-value = 0.101

Paired t-test:

Dependent Variable: Overall DBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.916)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	68.769	5.403	1.499
2.000	13	0	67.615	6.305	1.749
Difference	13	0	1.154	4.140	1.148

t = 1.005 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.348 to 3.656

Two-tailed P-value = 0.335

One-tailed P-value = 0.167

Paired t-test:

Dependent Variable: Awake DBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.771)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	74.231	4.833	1.340
2.000	13	0	72.692	6.183	1.715
Difference	13	0	1.538	5.364	1.488

t = 1.034 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.703 to 4.780

Two-tailed P-value = 0.321

One-tailed P-value = 0.161

Paired t-test:

Dependent Variable: Sleep DBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.732)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	13	0	57.692	6.701	1.858
2.000	13	0	58.000	7.561	2.097
Difference	13	0	-0.308	4.590	1.273

t = -0.242 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3.081 to 2.466

Two-tailed P-value = 0.813

One-tailed P-value = 0.407

One Way Repeated Measures Analysis of Variance

Dependent Variable: Glucose (mg/dl)

Normality Test (Shapiro-Wilk): Passed (P = 0.707)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.742)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	0	95.786	13.320	3.560
2.000	14	0	95.143	11.374	3.040
3.000	14	0	94.929	9.127	2.439
4.000	14	0	86.429	12.774	3.414

Source of Variation	DF	SS	MS	F	P
Between Subjects	13	5733.714	441.055		
Between Treatments	3	829.286	276.429	7.391	<0.001
Residual	39	1458.714	37.403		
Total	55	8021.714	145.849		

Power of performed test with alpha = 0.050: 0.952

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 4.000	9.357	4.048	0.001	Yes
1.000 vs. 3.000	0.857	0.371	1.000	No
1.000 vs. 2.000	0.643	0.278	1.000	Do Not Test
2.000 vs. 4.000	8.714	3.770	0.003	Yes
2.000 vs. 3.000	0.214	0.0927	1.000	Do Not Test
3.000 vs. 4.000	8.500	3.677	0.004	Yes

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.881)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1 to 2 (glucose)	14	0	-0.643	9.692	2.590
3 to 4 (glucose)	14	0	-8.500	7.230	1.932
Difference	14	0	7.857	11.635	3.109

t = 2.527 with 13 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 1.140 to 14.575

Two-tailed P-value = 0.0253

One-tailed P-value = 0.0126

One Way Repeated Measures Analysis of Variance

Dependent Variable: Ghrelin Concentration

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.074)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	1	185.259	156.800	43.489
2.000	14	1	170.127	151.810	42.104
3.000	13	0	146.911	125.559	34.824
4.000	14	1	180.686	210.067	58.262

Source of Variation	DF	SS	MS	F	P
Between Subjects	12	958427.530	79868.961		
Between Treatments	3	11412.893	3804.298	0.413	0.745
Residual	36	331879.211	9218.867		
Total	51	1301719.633	25523.914		

Power of performed test with alpha = 0.050: 0.050

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 3.000	38.348	1.018	1.000	No
1.000 vs. 2.000	15.132	0.402	1.000	Do Not Test
1.000 vs. 4.000	4.572	0.121	1.000	Do Not Test
4.000 vs. 3.000	33.775	0.897	1.000	Do Not Test
4.000 vs. 2.000	10.560	0.280	1.000	Do Not Test
2.000 vs. 3.000	23.215	0.616	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: Ghrelin Concentration

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Friedman Repeated Measures Analysis of Variance on Ranks

Dependent Variable: Ghrelin Concentration

Group	N	Missing	Median	25%	75%
1.000	13	1	144.611	63.677	359.728
2.000	13	1	115.953	59.785	335.404
3.000	13	1	107.461	40.237	302.234
4.000	13	0	123.913	52.444	213.251

Chi-square= 5.100 with 3 degrees of freedom. (P = 0.165)

Paired t-test:

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Ghrelin (1 to 2)	13	0	-15.132	36.546	10.136
Ghrelin (3 to 4)	13	0	33.775	164.280	45.563
Difference	13	0	-48.907	178.097	49.395

t = -0.990 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -156.530 to 58.715

Two-tailed P-value = 0.342

One-tailed P-value = 0.171

Paired t-test:

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Missing	Median	25%	75%
Ghrelin (1 to 2)	13	0	1.415	-46.526	14.506
Ghrelin (3 to 4)	13	0	-7.076	-53.956	48.649

W= 11.000 T+ = 51.000 T-= -40.000
Z-Statistic (based on positive ranks) = 0.384

P(est.)= 0.727 P(exact)= 0.735

One Way Repeated Measures Analysis of Variance

Dependent Variable: LEAP2 Concentration

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.777)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	14	1	0.818	1.884	0.522
2.000	14	1	0.129	0.911	0.253
3.000	13	1	0.749	1.439	0.416
4.000	14	1	0.415	1.239	0.344

Source of Variation	DF	SS	MS	F	P
Between Subjects	12	55.695	4.641		
Between Treatments	3	3.793	1.264	1.162	0.338
Residual	35	38.070	1.088		
Total	50	97.686	1.954		

Power of performed test with alpha = 0.050: 0.077

Expected Mean Squares:
Approximate DF Residual = 35.000
Expected MS(Subj) = var(res) + 8.513 var(Subj)
Expected MS(Treatment) = var(res) + var(Treatment)
Expected MS(Residual) = var(res)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
1.000 vs. 2.000	0.690	1.686	0.604	No
1.000 vs. 4.000	0.404	0.986	1.000	Do Not Test
1.000 vs. 3.000	0.0916	0.218	1.000	Do Not Test
3.000 vs. 2.000	0.598	1.423	0.981	Do Not Test
3.000 vs. 4.000	0.312	0.742	1.000	Do Not Test
4.000 vs. 2.000	0.286	0.700	1.000	Do Not Test

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.117)

Treatment Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
LEAP2 (1 to 2)	13	1	-0.710	1.510	0.436
LEAP2 (3 to 4)	13	1	-0.309	1.834	0.529
Difference	13	1	-0.401	1.608	0.464

t = -0.864 with 11 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.423 to 0.620

Two-tailed P-value = 0.406

One-tailed P-value = 0.203

One Way Repeated Measures Analysis of Variance

Dependent Variable: NPY Concentration

Normality Test (Shapiro-Wilk): Passed (P = 0.151)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.398)

Treatment Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
1.000	14	0	866.206	626.059	167.321
2.000	14	0	797.057	653.040	174.532
3.000	13	0	890.518	742.387	205.901
4.000	14	0	432.277	547.562	146.342

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Subjects	13	18998415.628	1461416.587		

Between Treatments	3	2057694.098	685898.033	12.110	<0.001
Residual	38	2152300.780	56639.494		
Total	54	23038680.434	426642.230		

Power of performed test with alpha = 0.050: 0.998

Expected Mean Squares:
Approximate DF Residual = 38.000
Expected MS(Subj) = var(res) + 3.923 var(Subj)
Expected MS(Treatment) = var(res) + var(Treatment)
Expected MS(Residual) = var(res)

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: **Visit**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
3.000 vs. 4.000	500.638	5.428	<0.001	Yes
3.000 vs. 2.000	135.858	1.473	0.894	No
3.000 vs. 1.000	66.709	0.723	1.000	Do Not Test
1.000 vs. 4.000	433.929	4.824	<0.001	Yes
1.000 vs. 2.000	69.149	0.769	1.000	Do Not Test
2.000 vs. 4.000	364.780	4.055	0.001	Yes

Paired t-test:

Normality Test (Shapiro-Wilk): Passed (P = 0.531)

Treatment Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
NPY (1 to 2)	13	0	-88.896	245.637	68.127
NPY (3 to 4)	13	0	-493.260	439.413	121.871
Difference	13	0	404.364	510.913	141.702

t = 2.854 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 95.622 to 713.106

Two-tailed P-value = 0.0145

One-tailed P-value = 0.00726

Table C.1.1: Results of post-hoc power analysis on 24-hour change data for key acute food deprivation variables

Results of Post-Hoc Power Analysis (Acute Food Deprivation)

	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Glucose (mg/dl)	Ghrelin (pg/ml)	LEAP2 (ng/ml)	NPY (pg/ml)
Mean 1 (control)	-2.79	-1.86	0.79	-0.64	-15.13	-0.71	-88.90
Sd 1 (control)	8.36	5.10	7.39	9.69	36.55	1.51	245.64
Mean 2 (treatment)	0.93	0.36	3.00	-8.50	33.78	-0.31	-493.26
Sd 2 (treatment)	4.75	2.92	5.68	7.23	164.28	1.83	439.41
Mean Difference	3.72	2.21	2.21	7.86	48.91	0.40	404.36
Effect Size	0.55	0.53	0.34	0.92	0.41	0.24	1.14
Sample Size	14	14	14	14	13	13	14
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Power (one-tailed)	0.62	0.60	0.33	0.95	0.40	0.20	0.99
Power (two-tailed)	0.47	0.45	0.21	0.89	0.28	0.12	0.97

C.2 Chronic Food Deprivation (Primary Outcome Variables)

One Way Repeated Measures Analysis of Variance

Dependent Variable: HR (bpm)

Normality Test (Shapiro-Wilk): Passed (P = 0.288)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.424)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	76.333	9.180	3.748
2.000	6	0	73.500	11.041	4.507
3.000	6	0	74.333	8.756	3.575

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	1144.278	228.856		
Between Treatments	2	25.444	12.722	0.471	0.637
Residual	10	269.889	26.989		
Total	17	1439.611	84.683		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 2.000	2.833	0.945	1.000	No
1.000 vs. 3.000	2.000	0.667	1.000	Do Not Test
3.000 vs. 2.000	0.833	0.278	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Data source: Hemodynamics Data in ACFD SigmaPlot Statistics (C3).JNB

Normality Test (Shapiro-Wilk): Passed (P = 0.130)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.271)

Treatment Name	N	Missing	Mean	Std Dev	SEM
HR (1 to 2)	6	0	-2.833	10.048	4.102
HR (2 to 3)	6	0	0.833	6.242	2.548
HR (1 to 3)	6	0	-2.000	4.690	1.915

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	146.667	29.333		
Between Treatments	2	44.333	22.167	0.334	0.724
Residual	10	663.000	66.300		
Total	17	854.000	50.235		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
HR (2 to 3) vs. HR (1 to 2)	3.667	0.780	1.000	No
HR (2 to 3) vs. HR (1 to 3)	2.833	0.603	1.000	Do Not Test

HR (1 to 3) vs. HR (1 to 2)	0.833	0.177	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: SBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.697)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.446)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	117.500	5.718	2.335
2.000	6	0	108.667	5.820	2.376
3.000	6	0	107.333	6.121	2.499

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	356.500	71.300		
Between Treatments	2	366.333	183.167	11.191	0.003
Residual	10	163.667	16.367		
Total	17	886.500	52.147		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 3.000	10.167	4.353	0.004	Yes
1.000 vs. 2.000	8.833	3.782	0.011	Yes
2.000 vs. 3.000	1.333	0.571	1.000	No

One Way Repeated Measures Analysis of Variance

Normality Test (Shapiro-Wilk): Passed (P = 0.622)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.273)

Treatment Name	N	Missing	Mean	Std Dev	SEM
SBP (1 to 2)	6	0	-8.833	4.355	1.778
SBP (2 to 3)	6	0	-1.333	5.888	2.404
SBP (1 to 3)	6	0	-10.167	6.676	2.725

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	297.111	59.422		
Between Treatments	2	272.111	136.056	7.017	0.012
Residual	10	193.889	19.389		
Total	17	763.111	44.889		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
SBP (2 to 3) vs. SBP (1 to 3)	8.833	3.475	0.018	Yes
SBP (2 to 3) vs. SBP (1 to 2)	7.500	2.950	0.044	Yes
SBP (1 to 2) vs. SBP (1 to 3)	1.333	0.524	1.000	No

One Way Repeated Measures Analysis of Variance

Dependent Variable: DBP (mmHg)

Normality Test (Shapiro-Wilk): Passed (P = 0.220)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.950)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	71.667	7.891	3.221
2.000	6	0	65.167	8.329	3.400
3.000	6	0	66.667	2.805	1.145

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	519.167	103.833		
Between Treatments	2	139.000	69.500	3.897	0.056
Residual	10	178.333	17.833		
Total	17	836.500	49.206		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 2.000	6.500	2.666	0.071	No
1.000 vs. 3.000	5.000	2.051	0.202	Do Not Test
3.000 vs. 2.000	1.500	0.615	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Normality Test (Shapiro-Wilk): Passed (P = 0.731)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.298)

Treatment Name	N	Missing	Mean	Std Dev	SEM
DBP (1 to 2)	6	0	-6.500	5.468	2.232
DBP (2 to 3)	6	0	1.500	6.091	2.487
DBP (1 to 3)	6	0	-5.000	6.325	2.582

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	266.667	53.333		
Between Treatments	2	217.000	108.500	4.043	0.052
Residual	10	268.333	26.833		
Total	17	752.000	44.235		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
DBP (2 to 3) vs. DBP (1 to 2)	8.000	2.675	0.070	No
DBP (2 to 3) vs. DBP (1 to 3)	6.500	2.173	0.165	Do Not Test
DBP (1 to 3) vs. DBP (1 to 2)	1.500	0.502	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: Glucose (mg/dl)

Normality Test (Shapiro-Wilk): Passed (P = 0.870)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.272)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	98.500	7.791	3.181
2.000	6	0	100.333	5.354	2.186
3.000	6	2	97.500	11.121	5.560

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	618.083	123.617		
Between Treatments	2	16.083	8.042	0.322	0.734
Residual	8	199.750	24.969		
Total	15	838.938	55.929		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
2.000 vs. 3.000	2.417	0.714	1.000	No
2.000 vs. 1.000	1.833	0.635	1.000	Do Not Test
1.000 vs. 3.000	0.583	0.172	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: Ghrelin (pg/ml)

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.528)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	89.417	85.407	34.867
2.000	6	0	91.833	86.187	35.186
3.000	6	0	159.647	127.294	51.968

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	102199.790	20439.958		
Between Treatments	2	19073.483	9536.741	1.819	0.212
Residual	10	52432.311	5243.231		
Total	17	173705.584	10217.976		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
3.000 vs. 1.000	70.230	1.680	0.372	No
3.000 vs. 2.000	67.813	1.622	0.408	Do Not Test
2.000 vs. 1.000	2.417	0.0578	1.000	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: Ghrelin (pg/ml)

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Friedman Repeated Measures Analysis of Variance on Ranks

Dependent Variable: Ghrelin (pg/ml)

Group	N	Missing	Median	25%	75%
1.000	6	0	67.835	31.213	137.710
2.000	6	0	81.100	25.818	152.838
3.000	6	0	142.310	37.672	302.672

Chi-square= 2.348 with 2 degrees of freedom. P(est.)= 0.309 P(exact)= 0.430

One Way Repeated Measures Analysis of Variance

Dependent Variable: LEAP2 (ng/ml)

Normality Test (Shapiro-Wilk): Passed (P = 0.442)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.965)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	0.615	0.902	0.368
2.000	6	0	0.295	0.638	0.261
3.000	6	0	0.705	0.802	0.327

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	7.436	1.487		
Between Treatments	2	0.557	0.279	1.476	0.274
Residual	10	1.888	0.189		
Total	17	9.881	0.581		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
3.000 vs. 2.000	0.410	1.634	0.400	No
3.000 vs. 1.000	0.0900	0.359	1.000	Do Not Test
1.000 vs. 2.000	0.320	1.276	0.693	Do Not Test

One Way Repeated Measures Analysis of Variance

Dependent Variable: NPY (pg/ml)

Normality Test (Shapiro-Wilk): Passed (P = 0.650)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.916)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	757.535	586.818	239.568
2.000	6	0	632.875	448.058	182.919
3.000	6	0	731.707	527.529	215.363

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	3917254.116	783450.823		

Between Treatments	2	51949.833	25974.917	1.300	0.315
Residual	10	199738.292	19973.829		
Total	17	4168942.242	245231.897		

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 2.000	124.660	1.528	0.473	No
1.000 vs. 3.000	25.828	0.317	1.000	Do Not Test
3.000 vs. 2.000	98.832	1.211	0.761	Do Not Test

Paired t-test:

Dependent Variable: HR

Normality Test (Shapiro-Wilk): Passed (P = 0.183)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	76.150	10.682	4.361
2.000	6	0	71.883	8.941	3.650
Difference	6	0	4.267	6.601	2.695

t = 1.583 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.661 to 11.194

Two-tailed P-value = 0.174

One-tailed P-value = 0.0871

Paired t-test:

Dependent Variable: RRI

Normality Test (Shapiro-Wilk): Passed (P = 0.489)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	803.833	104.358	42.604
2.000	6	0	849.500	113.417	46.302
Difference	6	0	-45.667	86.192	35.188

t = -1.298 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -136.120 to 44.786

Two-tailed P-value = 0.251

One-tailed P-value = 0.125

Paired t-test:

Dependent Variable: RRI-LF

Normality Test (Shapiro-Wilk): Passed (P = 0.573)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	606.167	691.928	282.478
2.000	6	0	581.333	831.027	339.265
Difference	6	0	24.833	782.745	319.554

t = 0.0777 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -796.607 to 846.274

Two-tailed P-value = 0.941

One-tailed P-value = 0.471

Paired t-test:

Dependent Variable: RRI-HF

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	1571.167	3062.849	1250.403
2.000	6	0	1239.333	1679.025	685.459
Difference	6	0	331.833	1486.593	606.899

t = 0.547 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1228.250 to 1891.917

Two-tailed P-value = 0.608

One-tailed P-value = 0.304

Paired t-test:

Dependent Variable: RRI-HF

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Wilcoxon Signed Rank Test

Dependent Variable: RRI-HF

Group	N	Missing	Median	25%	75%
1.000	6	0	332.500	257.250	2342.250
2.000	6	0	650.000	198.250	2066.250

W= 1.000 T+ = 11.000 T-= -10.000
Z-Statistic (based on positive ranks) = 0.105

Yates continuity correction option applied to calculations.

P(est.)= 1.000 P(exact)= 1.000

Paired t-test:

Dependent Variable: LF/HF

Normality Test (Shapiro-Wilk): Passed (P = 0.252)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	0.942	0.958	0.391
2.000	6	0	0.760	0.780	0.319
Difference	6	0	0.182	0.767	0.313

t = 0.580 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.623 to 0.986

Two-tailed P-value = 0.587

One-tailed P-value = 0.293

Paired t-test:

Dependent Variable: SAP

Normality Test (Shapiro-Wilk): Passed (P = 0.105)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	108.933	12.250	5.001
2.000	6	0	105.217	7.010	2.862
Difference	6	0	3.717	8.558	3.494

t = 1.064 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -5.265 to 12.698

Two-tailed P-value = 0.336

One-tailed P-value = 0.168

Paired t-test:

Dependent Variable: DAP

Normality Test (Shapiro-Wilk): Passed (P = 0.454)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	64.067	9.011	3.679
2.000	6	0	62.533	10.779	4.400
Difference	6	0	1.533	9.803	4.002

t = 0.383 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -8.754 to 11.821

Two-tailed P-value = 0.717

One-tailed P-value = 0.359

Paired t-test:

Dependent Variable: MAP

Normality Test (Shapiro-Wilk): Passed (P = 0.989)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	83.383	9.981	4.075
2.000	6	0	80.683	9.497	3.877
Difference	6	0	2.700	8.331	3.401

t = 0.794 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -6.043 to 11.443

Two-tailed P-value = 0.463

One-tailed P-value = 0.232

Table C.2.1: Results of post-hoc power analysis on pre/post data for key chronic food deprivation variables

Results of Post-Hoc Power Analysis (Chronic Food Deprivation)

	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Glucose (mg/dl)	Ghrelin (pg/ml)	LEAP2 (ng/ml)	NPY (pg/ml)
Mean 1 (pre)	117.50	71.67	76.33	98.50	89.42	0.62	757.53
Sd 1 (pre)	5.72	7.89	9.18	7.79	85.40	0.90	586.82
Mean 2 (post)	107.33	66.67	74.33	97.50	159.65	0.70	731.71
Sd 2 (post)	6.12	2.80	8.76	11.12	127.29	0.80	527.53
Mean Difference	10.17	5.00	2.00	1.00	70.23	0.09	25.83
Effect Size	1.72	0.84	0.22	0.10	0.65	0.10	0.05
Sample Size	6	6	6	6	6	6	6
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Power (one-tailed)	0.97	0.55	0.12	0.08	0.39	0.08	0.06
Power (two-tailed)	0.91	0.39	0.07	0.06	0.25	0.05	0.05

C.3 In-Depth Review

t-test

Normality Test (Shapiro-Wilk): Passed (P = 0.171)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.621)

Group Name	N	Missing	Mean	Std Dev	SEM
SBP Before Ramadan	3	0	127.933	5.278	3.047
SBP Ramadan Week 3	2	0	121.400	3.677	2.600

Difference of means 6.533

Equal Variances Assumed (Student's t-test):

t = 1.490 with 3 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -7.422 to 20.489

Two-tailed P-value = 0.233

One-tailed P-value = 0.117

Equal Variances Not Assumed (Welch's t-test):

t = 1.631 with 2.899 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -10.701 to 23.768

Two-tailed P-value = 0.205

One-tailed P-value = 0.102

t-test

Normality Test (Shapiro-Wilk): Passed (P = 0.835)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
DBP Before Ramadan	3	0	79.367	8.364	4.829
DBP Ramadan Week 3	2	0	75.600	1.980	1.400

Difference of means 3.767

Equal Variances Assumed (Student's t-test):

t = 0.596 with 3 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -16.350 to 23.883

Two-tailed P-value = 0.593

One-tailed P-value = 0.297

Equal Variances Not Assumed (Welch's t-test):

t = 0.749 with 2.318 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -17.867 to 25.401

Two-tailed P-value = 0.522

One-tailed P-value = 0.261

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t-test

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.539)

Group Name	N	Missing	Mean	Std Dev	SEM
HR 1hr Healthy	4	0	68.550	3.639	1.820
HR 12hr Healthy	4	0	66.575	9.512	4.756

Difference of means 1.975

Equal Variances Assumed (Student's t-test):

t = 0.388 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -10.485 to 14.435

Two-tailed P-value = 0.712

One-tailed P-value = 0.356

Equal Variances Not Assumed (Welch's t-test):

t = 0.388 with 3.860 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -12.368 to 16.318

Two-tailed P-value = 0.719

One-tailed P-value = 0.359

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t-test

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%

HR 1hr Healthy	4	0	66.850	66.550	72.250
HR 12hr Healthy	4	0	62.350	61.150	76.225

Mann-Whitney U Statistic= 4.000

T = 22.000 n(small)= 4 n(big)= 4 P(est.)= 0.312 P(exact)= 0.343