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Effects of Dietary Preference on the Experience of Anxiety, Depression and Acute Stress Response

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EFFECTS OF DIETARY PREFERENCE ON THE EXPERIENCE OF ANXIETY, DEPRESSION AND ACUTE STRESS RESPONSE

by

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ABSTRACT
EFFECTS OF DIETARY PREFERENCE ON THE
EXPERIENCE OF ANXIETY, DEPRESSION AND ACUTE STRESS RESPONSE

by

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Research has demonstrated that high saturated fat and low carbohydrate consumption may provide physiological benefit in the treatment of major neurological disorders, though not much research has explored if these benefits extend to the experience of anxiety, depression, stress and physical symptoms. The purpose of this study was to explore the relationship between fat consumption and negative emotional/physiological states (anxiety, depression, stress and physical symptoms). This study also explored how fat consumption can alter one's response to an acute stress. Participants completed an online survey indicating their food preferences and their responses to a number of self-report scales such as the Physical Symptoms Inventory (PSI), Perceived Stress Scale (PSS), Hospital Anxiety and Depression Scale (HADS) and Depression, Anxiety and Stress Scale (DASS21). From those that completed the online survey, participants were recruited to complete the lab portion of the study. Participants initially completed the Positive and Negative Affective Schedule (PANAS) and Brief Fear of Negative Evaluation (BFNE), they then completed the Montreal Imaging Stress Task and completed a follow up PANAS. Heart rate (HR), cardiac output (CO), stroke volume (SV) and skin conductance level (SCL) were recorded throughout the study. Following the study, high frequency heart rate variability (HF) and percentage of normal to normal

beats greater than 50 msec (pNN50) was calculated and participants were split into three groups based on saturated/mono-saturated fat consumption. It was found that participants who consumed of high amounts of saturated/mono-saturated fat demonstrated high HF and pNN50 values prior to the MIST, increased flexibility in response to the MIST and a return to baseline following the MIST. Increased fat consumption was also associated with lower depression scores (HADS) and lower baseline negative affect (PANAS). There was no significant change in HF or pNN50 for those who consumed a moderate amount of saturated/mono-saturated fat. For those participants who consumed a low amount of saturated/mono-saturated fat, there was a decrease in pNN50 during the MIST. Results indicate that increased fat consumption may have generated a more flexible and favorable autonomic state, while those who consumed low amounts of fat demonstrated a decrease in HRV during the MIST and minimal recovery back to baseline following the MIST.

DEDICATION

I would like to thank my advisor, Dr. Raymond Fleming, for his steadfast support and harrowing task of keeping me grounded as I've completed my doctoral journey. Ray has constantly given me thoughtful and challenging feedback, he has been pivotal in my growth as a researcher. I thank him for all the hours and late nights spent shaping me into the researcher I currently am, and hope I can do justice to the effort he has invested.

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CHAPTER I: REVIEW OF THE LITERATURE

Utilization of a high fat/low carbohydrate diet to induce a ketogenic state has been used to improve symptomology of a variety of treatment resistant neurological ailments such as epilepsy, autism and Alzheimer's (Masino, Kawamura, Wasser, Pomeroy & Ruskin, 2009; Politi, Shemer-Meiri, Shuper & Aharoni, 2011). The ketosis can be either induced through fasting or consumption of a predominantly high fat diet (saturated, monosaturated and omega 3 polyunsaturated fats). Utilizing a high fat diet has commonly been used as way of inducing a state of mild ketosis; a diet composing of 10% carbohydrate (50-100g minus fiber), 70% Sat/Monsaturated fat (unlimited) and 20% protein (.4g x body weight) are commonly used (Forsythe et al., 2008; Foster et al. 2003; Masino et al., 2009; Politi et al., 2011; Sato, et al., 1995; Volek, Fernandez, Feinman & Phinney, 2008; Volek et al., 2009; Volek et al., 2009).

High fat/Low Carbohydrate

The specific mechanisms by which high fat/low carbohydrate diets exert their benefits are up to debate; in general the limitation of carbohydrates causes the body to switch to burning fat as the preferred energy source. The liver maintains energy homeostasis by converting fat and some amino acids into ketone bodies (acetoacetate, β-hydroxybutyrate and acetone), which are then used as fuel instead of carbohydrates (Masino et al., 2009; Politi et al, 2011). Ketone bodies are converted to acetyl-CoA, which enters the mitochondrial tricarboxylic acid cycle, replacing pyruvate (derived from glycolysis). Metabolism of ketone bodies is also more efficient than that of glucose, leading to more available energy for synthesis of adenosine triphosphate (ATP). This is due to the higher heat combustion of ketone bodies as opposed to pyruvate. Ketone

bodies also readily pass through the blood brain barrier, while glucose requires a transporter (Veech, 2004). By means of the TCA cycle, Acetyl-CoA increases the amount of adenosine, ATP, γ-aminobutyric acid (GABA), glutamate and brain mitochondria (Masino, Kawamura, Wasser, Pomeroy & Ruskin, 2009). General cellular energy is also up-regulated. A ketogenic state has also been found to reduce production of reactive oxygen species (ROS), especially in the brain. The increases of these substrates are thought to provide the benefits observed from the ketogenic state.

Epilepsy

Induction of a ketogenic state has classically been used to treat drug resistant epilepsy and has been utilized since the 1920's, without negative long-term consequences (Freeman, 2006). The multiple neurophysiological changes that occur following induction of a ketogenic state are thought to be responsible for the anticonvulsant effects seen in participants. As a byproduct of increased ATP, there is also an increase in Adenosine A1 in the hippocampus and cerebral cortex, which has been found to produce anticonvulsant effects (Dunwiddie, 1999; Masino et al., 2009). Adenosine enacts a tonic modulatory influence on neural activity and is able to stop seizures that are pharmacologically resistant (Masino et al., 2009). There is also the effect of the ketones themselves upon the neurological milieu; β- hydroxybutyrate, acetone, and especially acetoacetate are the preferred neural substrates for neural lipid synthesis (Gaior et al., 2006; Pan, de Graaf, Petersen, Shulman, Hetherington & Rothman 2002; Pierre & Pellerin, 2005). This implies that increasing the amount of ketones in the brain intercellular fluid could assist with the production of a variety of white matter generating substrates and if so, this would have implications for the treatment/managment of a

number of other neurological diseases. What's also interesting are the changes that occur in children with drug resistant epilepsy, those who remained in a ketogenic state for more than a year had positive effects 3-6 years after initiation and it was found that 49% of children experienced a greater than 90% resolution of seizures (Marsh, et al., 2006).

Safety Concerns

Due to the current concerns about saturated fat, there are questions regarding the safety of a high fat/low carbohydrate diet. With regards to side effects, most of them have to do with the transition period that occurs when the body up regulates the necessary mechanisms to burn fat as the predominant fuel source. Side effects are short term and include hunger, constipation and lethargy, though these side effects are often the result of lost water weight during the adaptation process (Neal, et al., 2008). During the initial induction of a ketogenic diet, the body no longer conserves or retains water and when it releases this water, sodium and potassium accompany this loss (Phinney, 2004). Increased water, potassium and sodium intake can reduce or nullify most short-term side effects (Phinney, 2004; Phinney, Bistrian, Evans, Gervino & Blackburn, 1983; Phinney, Horton, Sims, Hanson, Danforth & Lagrange, 1980). The biggest safety concern has to do with the predominant ingestion of saturated fat and it's supposed connection to heart disease and metabolic syndrome. Current reviews have found this link to be questionable, and mostly based upon research has that has since been discredited due to design flaws, having to do with failure to control for type of fat consumed, amount of carbohydrate and other possible dietary moderators (i.e. smoking, drinking, carbohydrates consumed, consumption of processed meat products, types of fat consumed etc.) (Hoenselaar, 2012; Lawrence, 2013; Siri-Tarino, Sun, Hu & Krauss, 2010; Stanley, Dabkowski, Ribeiro &

O'Connell, 2012). These same reviews have found that replacing carbohydrates with a combination of fats (saturated, mono-saturated and Omega 3 fatty acids) actually stops the progression of metabolic syndrome and often improves disease states such as diabetes, atherosclerosis and cardiovascular disease.

Cardiovascular risk factors

With regards to cardiovascular disease, research has shown that most risk factors are actually improved on a diet that is predominantly saturated/mono-saturated fat (from dairy, coconut and animal protein sources). It's been consistently found that utilizing a high fat/low carbohydrate diet produces significantly decreased triglycerides, decreased VLDL, increased HDL, decreased total serum saturated fat, decreased ApoB/A, decreased leptin levels and increased brachial artery diameter (Foster et al., 2003; Forsythe et al., 2008; Volek, Fernandez, Feinman & Phinney, 2008; Volek et al., 2009; Volek et al., 2009). Forsythe and associates (2008) investigated obese men and women put on either a very low carbohydrate diet or a low fat diet, what they found was that there were decreases in multiple markers of inflammation. There were significant decreases in tumor necrosis factor alpha (NCF-a), interleukin 8 (IL8), monocyte chemotactic protein-1 (MCP 1), intracellular cellular adhesion molecule-1 (ICAM-1), E-selectin and plasma plasminogen-activator inhibiter-1 (PAI-1). Studies that have demonstrated an increased risk of heart disease, often fail to control for consumption of carbohydrates and will often fail to separate saturated fat from unsaturated fats (Standard American Diet SAD: trans fats, polyunsaturated fats and hydrogenated vegetable oils) (Atrup et al., 2012; Lawrence, 2013; Siri-Tarino et al., 2010).

Diabetic Risk factors

There are also decreases in diabetic risk factors associated with ketosis. Type 2 diabetics put on a high fat/low carbohydrate diet demonstrated significant decreases in Hemoglobin A1c, glucose, uric acid and a 41.6% decrease in Triglycerides (Gannon & Nuttall, 2004; Samaha, et al., 2003; Stern, et al., 2004; Yancy, Foy, Chalecki, Vernon & Westman, 2005). In one trial, Yancy and associates observed that medication was discontinued or reduced in 17/21 participants and out of the 4 with no change, 2 were not previously on medication for diabetes (2005). Weight loss is common, especially in type 2 diabetics, but as Gannon & Nuttall (2004) have demonstrated it is not required for the favorable effects seen in diabetic participants. After a 5 week, low carbohydrate diet designed not to induce weight loss (high protein as opposed to high fat), there was a significant decrease in plasma glucose, serum insulin, hemoglobin A1c, plasma glucagon and plasma triacylglycerol.

Effects of Diet on Depression and Anxiety

So, how might one's dietary composition affect one's experience of anxiety and depression? In a study looking at the response to consumption of a high carbohydrate meal compared to a high fat meal, it was found that after 3 hours a meal low in fat and high in carbohydrate was found to increase participants' report of feeling antagonistic, less friendly, less contented, less interested, less sociable and less outgoing, in comparison to ingesting a high fat meal (Wells & Read, 1996). Many studies looking at dietary intake have an issue with accuracy with regards to self-report of daily food consumption, it's often difficult to control what participants consume and to get an accurate record of it. As a follow up study, Wells, Read, Laugharne and Ahluwalia (1998) performed an 8-week trial in which self-report was not an issue since the research

group provided meals throughout the study. The study was also counter balanced based on meal type; one group was fed a medium fat diet, while the other was fed a low fat diet. The intervention began at week 4, with half the participants changing to a low-fat diet. Blood cholesterol and profile of mood states (POMS) were recorded, as well as other measures that did not show a significant change for either group. For the Low fat diet, there was a significant increase in anger-hostility, a nearly significant increase in depression-dejection and no change in other measures of the POMS. The medium fat group indicated a significant decrease in tension-anxiety. There was also a decrease in HDL cholesterol for the low fat diet, which is a *negative* marker of health. Participants in both groups reported that the diets were highly palatable and 2/3^{rds} of the low fat diet group could not tell the difference during the intervention. Indicating that most of the group that switched to low fat could not tell the difference between the medium fat meals they had been consuming.

The mechanism causing the change in affect in response to dietary changes may stem from a combination of factors that includes insulin reactivity, changes in blood glucose, serum levels of ghrelin, leptin and/or serum triglyceride levels. Ghrelin levels, which are inversely linked to plasma levels of leptin, have been found to blunt the sympathetic nervous system (Lambert, et al., 2011). Following administration of the Trier Social Stress Task, both lean and overweight men infused with ghrelin produced a decrease in mean blood pressure and there was also a decrease in mean sympathetic nerve activity. Under stress, ghrelin decreases sympathetic activity, while saline infusion demonstrated an increase in sympathetic activity. Ghrelin is just one of the hormones that are increased during both fasting and a ketogenic state. With regard to blood sugar,

Shoemaker and associates found that in a non-diabetic adolescent population depressive symptomology was associated with higher fasting insulin levels and decreased insulin sensitivity (2010). This association was significant even after controlling for fat mass, fat-free mass, height, age and other basic physical characteristics. Another study, looking at blood glucose variability in type II diabetics over a 72-hour period, found that participants with greater blood glucose variability (larger spikes and drops) reported significantly higher trait anxiety, depression and a decrease in overall quality of life (Penckofer et al, 2012).

With regards to how anxiety and depression are treated, Allaman, Fiumelli, Magistretti and Martin (2011) found that the two most commonly used SSRI's, Fluoxetine (Prozac) and paroxetine (Paxil), have been found to exert effects on a number of glucose mediated pathways. In rats infused with the medication, there was a significant up-regulation of brain derived neurotropic factor (BDNF), vascular endothelial growth factor (VEGF) and VGF mRNA expression. It was also found that Prozac and Paxil reduced glycogen levels, increased glucose utilization and increased the release of lactate by astrocytes. These 2 common SSRI's may exert their effect by regulating how glucose is metabolized. Therefore, a ketogenic diet high in fat, which positively affects blood sugar and insulin sensitivity, may also have similar effects on depression and anxiety

RNA expression in Anxiety and Depression

Individuals with anxiety and depression also seem to experience a cascade of changes in RNA expression affecting the metabolism of glucose (Gormanns et al., 2011). The expression of glycolysis phenotypes indicates a significant up-regulation for anxiety and a down regulation for depression, indicating an increase in sensitivity to

carbohydrates for anxiety and a decreased sensitivity for depression. There is also a slight down regulation of VEGF in both anxiety and depression. This pathway is essential in controlling permeability of endothelial cells and maintaining gap junctions, two key components in vascular permeability and maintenance of the blood brain barrier. There is also increased phenotypic expression in synaptic long-term potentiation (LTP), both depression and anxiety showed a slight down-regulation in LTP expression. With regards to anxiety, there is a significant up-regulation of phenotypes involved in the tricarboxylic acid cycle (TCA), along with the up-regulation in glycolysis. There is also a dysregulation of phenotype expression of tight junctions, which can specifically compromise the permeability of the blood brain barrier.

With regards to depression, there is a significant down-regulation in ubiquitin-mediated proteolysis, this pathway is responsible for the metabolism of protein and is also activated in response to cellular stress. Dysregulation in this pathway has been found in bipolar depression and schizophrenia (Konradi, Eaton, MacDonald, Walsh, Benes & Heckers, 2004; Bousman, et al., 2009). There is also a down regulation in gap junction phenotype expression and in combination with the down-regulated VEGF pathway; vascular permeability may be compromised in those with depression (Gormanns et al., 2011). A decrease in the ability to break down protein and a decrease in vascular permeability, could allow undigested proteins to enter the vasculature, causing possible abnormal blood brain communication. This could also account for the observed dysregulation in phenotype expression in LTP in both anxiety and depression (Gormanns et al., 2011; Shalev, Serlin & Friedman, 2009). Overall, this implies that there could be overexpression/dysregulation of phenotypes involved in carbohydrate metabolism and an

increase in gut, vascular and blood brain barrier permeability in those with anxiety and depression.

Leptin

There is sparse research investigating whether ketosis could be used for decreasing the symptoms of anxiety and depression, most work has been done with animal models, but there are some common findings that could be applicable to a human population. A diet designed to induce ketosis increased open arm entries in the elevated plus maze and also increased exploration of a novel object, while inducing a reduction in acetylcholinesterase. This indicates that the high fat diet may increase neurotransmission via acetylcholine in the frontal cortex and hypothalamus (Morganstern, Ye, Liang, Fagan, & Leibowitz, 2012). Diets high in fat, but not high in carbohydrates or protein, were anxiolytic after just one week of intervention (Murphy & Mercer, 2013; Prasadand & Prasad, 1996). Source of fat is also important and any type will not do; saturated fat appears to be anxiolytic, while trans fats and poly-unsaturated fat tend to be anxiogenic (Murphy et al., 2013; Teixeira, et al., 2011). The common element between utilizing a high fat diet to induce ketosis and the effects of medication seems to be a combination of plasma leptin levels and leptin sensitivity.

It has been found that Leptin has a similar effect as Prozac for treating depression and anxiety (Liu, Garza, Bronner, Kim, Zhang & Lu, 2010). Acute arterial administration of either Prozac or leptin produced equivalent antidepressant effects in mice. There was also a significant increase in mobility time during a tail suspension test and a forced swim test after administration of Prozac and leptin compared to mice administered saline. In contrast, administration of Prozac elicited anxiogenic effects, while leptin elicited

anxiolytic effects. Mice administered Prozac spent significantly less time in the open arm segments of an elevated plus maze and entered the open areas significantly less than mice administered a saline solution. Mice administered leptin spent significantly more time in the open area of the elevated plus maze and entered the open area significantly more times than either mice administered saline or Prozac. With regards to prosocial behavior, mice administered Prozac were significantly less social than mice administered either the saline or leptin, while mice administered leptin demonstrated significant increases in prosocial behavior. Mice administered Prozac demonstrated a significant decrease in total interaction time with other mice; a decrease in time spent nosing, following and amount of time in physical contact. Mice administered leptin demonstrated a significant increase in time spent nosing, following and a significant decrease in time spent self-grooming.

Triglycerides

Resistance to the antidepressant and anxiolytic effects of leptin could be a major source of many symptoms associated with anxiety and depression. Many of the refined carbohydrates and hydrogenated fats individuals ingest on the standard American diet can increase triglyceride levels. Banks and Farrell (2003) demonstrated that increased levels of triglycerides impair the ability of leptin to cross the blood brain barrier and exert its effects upon the hypothalamus, and is also the likely cause of leptin resistance. Researchers are just beginning to learn how important leptin resistance is with regards to the progression of metabolic syndrome. Obesity in humans and rat models were consistently associated with a resistance to, but not a deficiency in letpin (Considine, et al., 1996; Heymsfield, et al.,1999; Banks, DiPalma & Farrell, 1999). It was also found that when triglyceride levels were increased by food or starvation (48 hour fast), leptin

transport across the blood brain barrier was significantly decreased. When intralipid injection was introduced and triglyceride levels decreased, leptin transport was significantly increased. A 15 hour fasting state was also found to significantly decrease triglycerides and increase leptin transport. Overall, the study demonstrated that the greater the decrease in triglycerides, the more leptin that was transported across the blood brain barrier (Banks, et al., 2004).

One of the most common findings in studies that have induced ketosis is that serum triglyceride levels are often decreased and some metabolic benefits found during this state are thought to result from an increase in leptin sensitivity. Being in ketosis decreases triglycerides, which should make the brain more permeable and sensitive to leptin, ghrelin, insulin, serotonin, as well as others (Forsythe et al., 2008; Foster et al., 2003; Volek et al., 2008; Volek et al., 2009; Volek et al., 2009). Over time though, a ketogenic diet in humans decreases serum leptin and insulin levels, while in animals it increases leptin levels (Jenkins, Markovic, Fleury & Campbell, 1997). Therefore humans may need a "boost" in leptin levels over time to maintain the benefits of increased leptin sensitivity, while avoiding the pathologies that develop from the loss of leptin and insulin sensitivity.

It has also been found that sympathetic activity can impact serum leptin levels and leptin sensitivity. Aggel-Leijssen, Van Baak, Tenenbaum, Campfield and Saris (1999) measured how changes in various environmental responses affect plasma leptin, with carbohydrates being one of the variables. Plasma leptin is unchanged by light exercise, but is significantly decreased by strenuous acute exercise. During the study, some participants received an infusion of IL-6 or epinephrine to determine what may be

causing the decrease in leptin after intense acute exercise (Keller, Keller, Steensberg, Robinson and Pederson, 2005). It was found that epinephrine, but not IL-6 produced a significant decrease in leptin. It was also found that when acute exercise was combined with carbohydrate consumption, there was no post-exercise drop in plasma leptin. The largest change was in the exercise/carbohydrate group, which was an increase in glucose and insulin. This change in glucose metabolism, also decreased the plasma levels of epinephrine and IL-6 compared to exercise alone. In combination with epinephrine being released during stress and its ability to lower plasma leptin (known to produce feelings of satiety), this provides a plausible explanation for why individuals may choose to overeat carbohydrates when under stress.

Autonomic Response

While it has been demonstrated that consuming carbohydrates after intense exercise and fasting can ameliorate the release of epinephrine and maintain serum leptin and insulin, consuming processed carbohydrates (away from exercise) generally increases sympathetic activity. Intake of carbohydrates, but not fat or protein, has been found to increase SNS activity (Tentolouris, et al., 2003; Webbe, Libavivat & Campbell, 1981; Welle, 1995). Glucose alone has been found to increase secretion of norepinephrine, while fasting has been found to have an inhibitory effect on the SNS. Fat and protein seem to not affect the SNS/PNS balance. Infusion of β-hydroxybutyrate has been shown to blunt or decrease sympathetic activation. It was found to decrease HR, inhibit firing in cultured sympathetic neurons and decrease plasma norepinephrine (Kimura, et al., 2011).

Body weight also influences how reactive one's sympathetic response to carbohydrates is, Tentolouris and associates (2003) found that in lean female participants

fed a high carbohydrate diet, there was a significant increase in plasma norepinephrine levels, an increase in blood glucose and an increase in insulin. With regards to heart rate variability, there was an increase in low to high frequency ratio of heart rate variability (LF/HF ratio), increase in low frequency HRV (LF) and a decrease in high frequency HRV (HF), indicating sympathetic dominance and a significant decrease in overall heart rate variability. In lean women fed a high fat meal, there were no significant changes, with the exception of a significant decrease in blood glucose. Obese female participants indicated no significant changes in norepinephrine, nor were there any changes in HRV measures, whether they were fed a carbohydrate rich meal or a high fat meal. The only observed change was an increase in blood glucose and insulin after the carbohydrate rich meal. These findings indicate that carbohydrates have a uniquely stimulating effect upon the sympathetic branch of the HRV spectrum, which is not seen with other macronutrients. According to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996), changes in the spectral power of HRV indicate variations in the symapthovagal influence over the heart. Typically a fast Fourier transform is run on a 5 min period or greater in order to accumulate changes in inter-beat intervals (differences between R-spikes of the QRS complex) of the heart. Slow or low frequency changes (.04-.15 Hz) are thought to be a combination of sympathetic and parasympathetic influences, while faster or high frequency changes (.15-.4) are thought to purely represent parasympathetic influences or vagal control over hear rate changes. The LF/HF ratio is thought to represent changes in the sympathovagal balance, though there is doubt as to whether LF or the LF/HF ratio

should be reported, since they reflect parasympathetic influences as well (Appelhans, & Leucken, 2006).

There are other findings that corroborate the previously mentioned blunting response as well (Jones et al., 2011; Jones et al. 2012). Jones and associates (2012) utilized a mental arithmetic task known as the Montreal Imaging Stress Task (MIST) to induce a stress state. They measured the differences in centrally mediated cardiovascular responses in those with low and high amounts of adipose fat. The researchers found that HR, cardiac output (CO), stroke volume (SV), blood pressure responses and cortisol responses were significantly blunted during the stress task compared to non-overweight participants, there was also an accompanying decrease in cognitive ability during the task. A lack of sensitivity to insulin and leptin could be contributing to this blunted response, which could also require obese participants to consume more carbohydrate in order to get an equivalent response as lean participants. Though all participants did indicate increases in HR, CO, SV and blood pressure when exposed to the MIST, which was found in a previous study performed by Jones and associates (2011). The participants in the 2 previous studies indicated a myocardial or beta-adrenergic response pattern to the MIST, indicating that they perceived the MIST as a surmountable challenge (Blascovich, Mendes, Tomaka, Salomon, & Seery 2003; Lawler, Wilcox, & Anderson, 1995; Prkachin, Mills, Zwaal, & Husted, 2001; Sherwood, Dolan, & Light, 1990). In contrasts, participants may also indicate a vascular or alpha-adrenergic response pattern to stress, which entails minimal change in CO, decreases in SV and total peripheral resistance (TPR).

If participants from Jones and associates' (2011; 2012) studies indicated a vascular response, they may have perceived the MIST as a threat and the participants may have subsequently performed much worse on the task (Blascovich, et al., 2003; Lawler, et al., 1995; Prkachin, et al., 2001; Sherwood, et al., 1990). With regards to depression and anxiety, it was found that depressed participants demonstrated a significant increase in vascular resistance when presented with an acute stress, indicating a relationship between vascular resistance and depression (Matthews, Nelsen, & Dimsdale, 2005). It was also found that increases in tension-anxiety and fatigue-inertia subscales of the POMS were associated with significant decreases SV (Yu, Nelesen, Ziegler, Dimsdale, 2001). There was also a further decrease in CO and an increase in TPR, but only for fatigue-inertia. These results provide evidence that anxiety and depression are associated with a hemodynamic profile that indicates a vascular response.

The autonomic system is also important in regulating the amount/type of serum lipid produced and how much is released into the blood stream. Parasympathetic efferent communication with the liver is required to limit the release of triglycerides into the blood stream, especially VLDL (BruinStroop, et al., 2013; Bruinstroop, et al., 2012). Rats that had their liver parasympathically denervated, produced a significant increase in plasma triglyceride compared to controls. Denervation of the sympathetic efferent activity to the liver did not produce the same amount of triglyceride release. As demonstrated previously, induction of a ketogenic state produces lower triglycerides, decreased serum glucose, a decrease in inflammatory markers, an up-regulation of cellular energy, decreased production of ROS, increased leptin/insulin sensitivity, marginally increased ghrelin, decreased hostility and decreased anxiety and depression in

animal models (Forsythe et al., 2008; Foster et al. 2003; Masino et al., 2009; Politi et al., 2011; Sato, et al., 1995; Volek, Fernandez, Feinman & Phinney, 2008; Volek et al., 2009; Volek et al., 2009). Since the physiological changes that accompany ketosis seem to also correlate to changes in autonomic functioning, it stands to reason that a high fat/low carbohydrate diet could make individuals more resilient to stress and less sympathetically reactive in general.

Research has shown that a state of ketosis is useful for diminishing multiple risk factors thought to exacerbate and/or cause diabetes, cardiovascular disease and epilepsy. It has been demonstrated to decrease triglycerides, improve healthy HDL:LDL ratios, modulate ghrelin, as well as increase both leptin and insulin sensitivity. Changes in carbohydrate metabolism, triglycerides, leptin and insulin have been found to impact the experience of depression and anxiety, though there is no current research that has sought to determine how ketosis could modify one's experience of anxiety and depression. The study that has come closest to this was performed by Wells and associates (1998), in which they provided food over an 8-week period. They found that low fat meals produced an increase in anger-hostility and a nearly significant increase in depression-dejection compared to medium fat meals. What the study didn't do was measure blood levels of ketones, which could have indicated a mechanism for the observed improvements in mood states. They also failed to determine if the participants' resilience to stress had been altered, or record electrophysiological measures to determine non-subjective improvements in reactivity to stress. Considering the number of physiological risk factors that a state of ketosis improves, it is surprising that researchers have yet to

thoroughly analyze its effects upon the symptoms of anxiety, depression, and reactivity to an acute stress.

The current study used self-report to measure subjective anxiety, depression and stress, it also used physiological measurements in combination with the MIST to determine if reactivity to stress is altered based upon whether participants are consuming high saturated/mono-saturated fat, moderate of saturated/mono-saturated fat or low saturated/mono-saturated fat. It was hypothesized that:

1. Participants consuming high saturated/mono-saturated fat will have fewer reported symptoms of anxiety and depression, perceived stress and physical symptoms than those consuming low or moderate saturated/mono-saturated fat.
2. Participants consuming moderate or low saturated/mono-saturated fat will show a more reactive stress response to an acute stress than those consuming a high saturated/mono-saturated fat, as evidenced by increases in skin conductance levels (SCL), heart rate (HR) and a decreases in pNN50 and HF.
3. Participants consuming a moderate saturated/mono-saturated fat or low saturated/mono-saturated fat will also demonstrate hemodynamic changes that indicate a vascular response to the stressor (minimal changes in SV and decreased CO), while participants consuming a high saturated/mono-saturated fat will demonstrate a myocardial response to the stressor (increased SV and increased CO).
4. Participants consuming high saturated/mono-saturated fat will also produce the most pronounced ketogenic state as demonstrated by increases in serum β-hydroxybutyrate compared to participants consuming moderate or low saturated/mono-

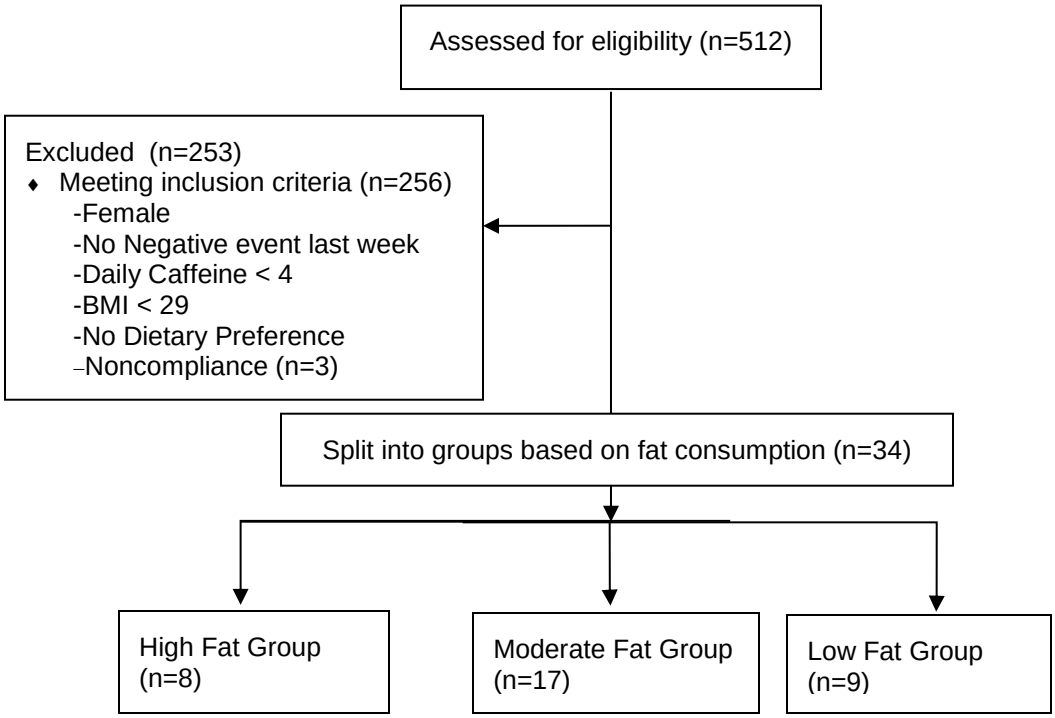
saturated fat. Differences between consumption of moderate and low saturated/mono-saturated fat diet are not likely to be significant.

CHAPTER II: METHODS

Participants

All participants N=512 completed an initial online survey that consisted of food preferences, a demographic/general health history survey, depression, anxiety, stress and physical symptom scales. Participants were excluded from selection for the laboratory portion of the study if they were male, indicated that they had experienced a negative event during the prior week, consumed more than four 8oz caffeinated beverages (coffee or energy drinks) per day, had a BMI of less than 29 and indicated no dietary preference (vegetarian, vegan, paleo, Atkins, etc.). Female participants N=256 were recruited from psychology courses to complete an online survey in exchange for extra credit A total of 34 female participants completed the laboratory portion of the study.

Table 1. *Consort Table*



Materials

Dietary preferences

A List of dietary preferences was used to determine how much participants identified with eating common processed carbohydrates alpha = .671 (bread, soda, candy, sports drinks, fruit juice, cereal and bagels), poly-unsaturated fat (vegetable oils), saturated and mono-saturated fats alpha = .658 (full fat dairy, cheese, cream cheese, butter, olive oil and coconut oil) and protein alpha = .655 (eggs, chicken, beef and fish). The scale measured "How often do you consume the following?" and ranged from '1 rarely' up to '5 often'.

Depression Anxiety and Stress Scale (DASS21)

The DASS 21 developed by Lovibond and Lovibond (1995), was designed to measure symptoms of depression, anxiety and stress. The depression subscale has been found to negatively correlate with low positive affectivity, the anxiety subscale was developed to indicate hyper-arousal or physical symptoms of anxiety, while the stress component has been found to positively correlate with the negative affectivity (Henry, & Crawford, 2005). Responses to the DASS21 questions were on a 4-point scale ranging from '0 Did not apply to me at all' up to '3 Applied to me very much, or most of the time' The reliability alphas of the 3 subscales has been found to be .88 for depression, .82 for anxiety and .90 for stress, indicating acceptable levels of reliability.

Hospital Anxiety and Depression scale (HADS)

The HADS scale was designed by Zigmoid and Snaith (1983) to determine severity of the symptoms of anxiety and depression separate from the somatic complaints

associated with these disorders. Inclusion of the HADS allows for a focus on the psychological symptoms of depression and especially anxiety. With regards to anxiety, the DASS21 focuses solely on the somatic symptoms of anxiety (Henry, & Crawford, 2005). Responses to the HADS questions were on a 4-point scale ranging from 0 up to 3, the meaning of the points on the scale varied depending on the question. The HADS has been utilized extensively in research for years and a review of those articles has found a reliability alpha of .85 or greater for both the anxiety and depression subscales (Bjelland, Dahl, Haug, & Neckelmann. 2002).

Physical Symptoms Index (PSI)

The PSI is a 12-item scale that measures how often one has experienced somatic complaints over the last month, complaints measured were primarily digestive symptoms (upset stomach, indigestion, constipation etc.) and neurological symptoms (headache, eye strain, dizziness, fatigue etc.) (Spector, & Jex, 1998). Responses to items on the PSI were based on a 5-point scale ranging from '1 Not at all' up to '5 Everyday'. The scale has been found to be reliable with an alpha of .82 and has also been to be a good indicator of physical symptoms resulting from stress, anxiety and depression (Spector, & Jex, 1998; Makikangas, & Kinnunen, 2003).

Perceived Stress Scale (PSS)

The PSS is a 14-item scale measuring how often an individual appraises the situations in their life as stressful (Cohenm Kamarck, Mermelstein, 1983). Participants were asked to indicate how often they felt a certain way or appraised a situation as within their control within the previous month, responses to the PSS were on a 5-point scale and ranged from'0 Never' up to '4 Very Often'. Appraisal of an event as stressful was found

to be a better predictor of physical symptoms resulting from stress, depression and anxiety than number/impact of negative life events. The PSS has been found to be highly reliable with an alpha of .86.

Brief Fear of Negative Evaluation (Brief FNE)

One of the main components of the MIST is negative evaluation of participant performance and therefore, it makes sense to control for oversensitivity towards negative social evaluation (Dedovic, et al., 2005). The brief FNE is a short 12-item scale that has been found to positively correlate with social avoidance and distress (Carleton, McCreary, Norton, & Asmundson, 2006). The scale for the bried FNE was a 5-point scale ranging from '1 Not at all characteristic of me' up to '5 Extremely characteristic of me'. The brief FNE has also been used in conjunction with anxiety scales to identify participants that may be suffering from an anxiety disorder and could be used to identify participants who would not be fit to include in the final analysis of the current study. The scale has also been found to be reliable with an alpha of .89.

Positive and Negative Affective Schedule (PANAS)

The PANAS is a 20-item scale designed to measure positive (10-items) and negative (10-items) affect (Crawford, & Henry, 2004). The 20 items of the PANAS are single words representing a variety of positive and negative mood states. The scale is a 5-point scale ranging from 1 'very slightly or not at all' up to 5 'very much'. The positive affective scale has been found to negatively correlate with DASS anxiety, DASS depression, DASS stress, HADS anxiety and HADS depression, the negative affective scale has been found to positively correlate with the aforementioned scales as well. The

PANAS scale has been found to be reliable with an alpha of .89 for the positive affective scale and an alpha of .85 for the negative affective scale.

Physiological Measures

Physiological measures were recorded with the Biopac MP35 data acquisition device (Biopac Systems, Goleta, CA). A lead II configuration sampled at 500 Hz was utilized to record electrocardiogram (ECG). Electrodes were placed under the participant's right collarbone and left ankle, the grounding wire was not connected because it is included with the electrodes for impedance transducers. Biopac Student Lab Pro 4.0 (BSL) was used to automatically calculate heart rate (HR) and R-R intervals from the raw ECG waveform. The SS31L noninvasive cardiac output sensor were used to measure impedance cardiography (Zo, dZ/dt and $dZ/dt_{(max)}$) and were recorded with strip electrodes placed on the participants neck (carotid artery) and mid/lower back (at heart level). Raw Z_0 (thoracic impedance) and dZ/dt (change in impedance over time) were recorded, these waveforms were used to calculate SV and CO. The Kubicek equation for calculating SV was utilized, $SV = (147*(L^2/Zo^2) \times T \times dZ/dt_{(max)})$ and CO (HR multiplied by SV) (Bernstein, & Lemmens, 2005). Skin conductance levels were recorded with electrodes placed on the first and middle finger of the left hand. After extracting R-R intervals from the raw ECG waveform, the percentage of normal-to-normal R intervals greater than 50 msec (pNN50) and the HF were calculated with Kubios HRV software 2.1 (Biosignal Analysis and Medical Imaging Group, Finland). Both the pNN50 and HF spectral power are thought to be more pure indices of vagal influence over the heart (Task Force, 1996). The "Precision Xtra Blood Glucose and Ketone Monitoring System" was used to measure serum levels of β-hydroxybutyrate.

Computerized Arithmetic Task

The MIST is a mental arithmetic stressor that has been adapted from the Trier Social Stress Task and has been reliably shown to elicit a physiological stress response (Dedovic, et al., 2005). The MIST is a computer program that combines mental arithmetic and negative social evaluations. The participant is acclimated to the task by being introduced to a five-minute "no pressure" trial, allowing them to learn how the answer dial works and how to enter responses. The "pressured" version of the task consists of a beeping timing bar and a feedback bar above the screen that indicates how the participant's performance stacks up to the average score of their peers. There are 3 zones that indicate level of performance and go from below average, average and above average (red, yellow and green). There is a "negative" sound for when participants get an answer wrong and a higher pitch "positive" sound when participants get the answer right. The math questions are a combination of addition, subtraction, multiplication and division. The program is designed to adjust to the participant's skill level and prevent them from scoring above average (yellow zone), it also prevents them from completely failing and disengaging from the task (deep into the red zone). If the participant gets three consecutive questions correct, the time allotted to answer the question decreases by 10% and if the participant misses 3 consecutive questions, the time allotted to answer the question increases by 10%.

Procedure

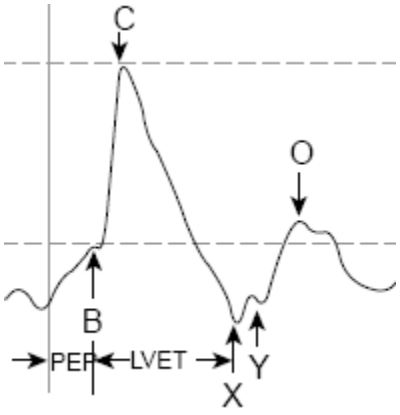
Prior to coming into the lab, participants were split into 3 possible groups following an exploratory analysis based upon their saturated/mono-saturated fat intake (reported prior to the lab portion). Participants whose fat intake exceeded the 75

percentile (56.25%) were assigned to the *high fat group*. Participants whose fat intake was between the 75th and 25th percentile (from 26.25% to 37.5%) were assigned to the *moderate fat group*. Participants whose fat intake was less than the 25th percentile (37.5%) were assigned to the *low fat group*. Neither the researcher nor the participant knew how the groups were split or which group the participant belonged to. The lab experience was also identical for each participant. Only after all participants were run, were their physiological data matched up with their pre-screening surveys and analyzed, making this study a double blind research design.

Once the participant arrived, they were instructed to have a seat and read through a consent form. After obtaining informed the consent, the researcher began the experiment. The participant's name was then recorded into a coding book, to keep their information confidential. A drop of blood was then drawn to test for the amount of serum β-hydroxybutyrate being produced by the participant. The participant was given the option to use the lancet themselves or have the researcher operate it, in most instances the participant preferred the researcher to activate the lancet. Following the ketone test, physiological electrodes and transducers were attached. The distance between thoracic impedance electrodes was measured in centimeters for later entry into the SV equation. Once the electrodes were connected, a 30 sec collection period was recorded and from this period, the average left ventricular ejection time (LVET) of the participant was calculated from 10 clean heart beats. The researcher measured from the B-point (systolic ventricular ejection) of the impedance waveform to the X-point (end systolic ventricular ejection) and recorded how much time it took for all blood to be ejected from the left ventricle (figure 1). The distance between impedance electrodes and the average LVET

were then input into the Kubicek equation, from this point CV and CO were automatically calculated through Biopac Student Lab Pro 4.0.

Figure 1. *Impedance waveform*



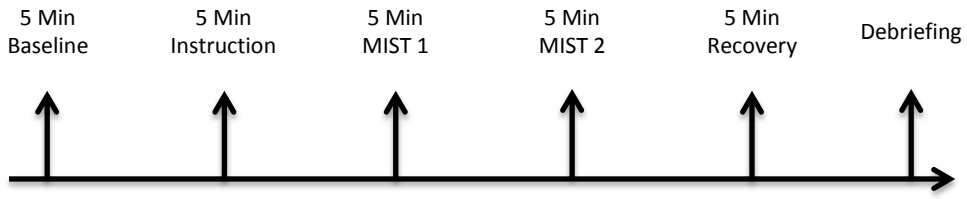
The researcher measured from the B-point (systolic ventricular ejection) of the impedance waveform to the X-point (end systolic ventricular ejection) and recorded how much time it took for all blood to be ejected from the left ventricle (figure 1). The distance between impedance electrodes and the average LVET were then input into the Kubicek equation, from this point CV and CO were automatically calculated through Biopac Student Lab Pro 4.0.

Prior to starting the MIST, a 5-min baseline was then recorded. The participant was directed to relax and remain still to prevent noise in the physiological recording (figure 2). Following the baseline, the researcher began the instruction period of the MIST and the participant was instructed on how to use the move the answer dial and choose their responses. The questions during the instruction period were relatively simple (smaller integers, no fractions) so the participant could become acclimated to the program interface while experiencing minimal stress. The next 5-min of the MIST was the pressured task (experimental period) without social feedback. As described previously, the participant was told that their performance was being compared to the performance of

their peers and if they do not perform adequately (stay within the yellow bar), their data will not be usable and will have to repeat the condition. All participants were told their performance was not good enough and that they must repeat the task. Following the completion of the second pressured task, a 5-min recovery period was recorded during which participants were instructed to relax and remain still to minimize noise in the physiological recordings.

After completion of the MIST, all electrodes and transducers were removed from the participant. The participant then completed a second PANAS and also completed a 3 day food journal. At the conclusion of the study, the participant was asked a series of debriefing questions about their diet and the purpose of the MIST. The researcher then informed the participant that their performance on the MIST was not being compared to their peers and that this was a manipulation to increase effort and elicit a stress response. The participant was then allowed to ask questions and bring any issues they had to the researcher's attention.

Figure 2. *Timeline*



Data Analysis

Statistical analyses were run using SPSS (version 21). Partial correlations were run to determine relationships between dietary preference, depression, anxiety, stress and somatic symptoms. BMI, caffeine consumption, exercise frequency and exercise exertion were entered as covariates because it has been found that higher values of BMI have been found to blunt autonomic responses and negatively affect participants' report of anxiety

and depression symptoms (Banks, DiPalma & Farrell, 1999: Considine, et al., 1996; Heymsfield, et al.,1999; Jones et al., 2011; Jones et al. 2012). Higher levels of caffeine consumption have also been found to increase reported symptoms of anxiety, depression and hostility (Veleber & Templer, 1984). In contrast to BMI and caffeine consumption, exercise has been found to exert anxiolytic, anti-depressant and more favorable mood states in general (Byrne & Byrne, 1993). The current study seeks to discover if dietary preference exerts an affect above and beyond that of BMI, Caffeine, and exercise. Linear regressions were then run for any significant partial correlations to extract R^2 change scores and Beta values.

Partial correlations were also run for those participants who completed the lab portion to determine the relationship between serum β-hydroxybutyrate, dietary preferences, depression, anxiety, stress and somatic complaints, with BMI caffeine consumption, exercise frequency and exercise exertion as covariates. Linear regressions were then run for any significant partial correlations to extract R^2 change scores and Beta values. Partial correlations were also run to determine to what degree serum levels of β-hydroxybutyrate predicts change in depression, anxiety, stress and somatic symptoms, with BMI caffeine consumption, exercise frequency and exercise exertion as covariates. Linear regressions were then run for any significant partial correlations to extract R^2 change scores and Beta values. Partial correlations were also run to determine to what degree dietary preferences predict differences in baseline physiology, briefFNE and PANAS values, with BMI caffeine consumption, exercise frequency and exercise exertion as covariates. Linear regressions were then run for any significant partial correlations to extract R^2 change scores and Beta values.

Multiple repeated measures ANOVA (time X group) were run to analyze changes in physiology (SCL, HR, SV, CO, pNN50 and HF) from instruction to the post-MIST recovery period, with diet group as a between subjects factor. Repeated measures ANOVAs were also run to analyze changes in the positive and negative affect of participants from baseline to after the MIST. Significant time by group interactions were followed up with repeated measures ANOVA split by group, least significant difference post hoc tests, and t-tests.

CHAPTER III: RESULTS

Grouping Variable

Results indicated significant group differences in the consumption of saturated/mono-saturated fat $F(2,31)=34.071$, $p<.001$, $\eta^2=.687$ and protein $F(2,31)=6.826$, $p<.01$. Simple effects tests were run as a follow-up and found that participants in the high fat group consumed more saturated/mono-saturated fats $t(23)=3.534$, $p<.001$ and protein $t(11.568)=2.824$, $p<.05$ than those in the moderate fat group. Participants in the high fat group consumed more saturated/mono-saturated fats $t(13.533)=6.152$, $p<.001$ and protein $t(14.903)=3.192$, $p<.01$ than the low fat group, and those in the moderate fat group also consumed more saturated/mono-saturated fat than the low fat group $t(24)=6.573$, $p<.001$. There were no differences in food preference for carbohydrates or poly-unsaturated fats between groups.

Table 1

Demographics and Descriptive Statistics

	Online N=256		Lab N=34					
			High Fat N=8		Mod Fat N=17		Low Fat N=9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	20.64	3.355	22	3.422	23.823	9.573	21.22	2.682
BMI	22.17	2.491	22.2	1.207	24.756	5.969	23.17	1.38
Ethnicity	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Native American	3	1.2	0	0	1	5.9	0	0
Asian American	21	8.2	0	0	1	5.9	0	0
Africa American	11	4.3	0	0	1	5.9	0	0
Hispanic	18	7	2	25	1	5.9	1	11.1
Caucasian	194	75.8	6	75	12	70.6	8	88.9
Other	9	3.5	0	0	1	5.9	0	0

Abbreviations: BMI=Body Mass Index

Survey Analyses

For all survey respondents eligible to participate in the lab portion of the current study, regression analyses were run to determine if food preference was associated with scores on the self-report scales (PSI, PSS, HADS-A, HADS-D, DASS21-A, DASS21-D and DASS21-S). All regression analyses controlled for BMI, caffeine consumption, exercise frequency and exercise exertion. Consumption of saturated/mono-saturated fat was significantly associated with lower HADS depression scores $\beta = -2.411$, $t(250)=-2.106$ $p=.088$. Regression results indicate that consumption of poly-unsaturated fats accounted for 1.6% (R^2change=.016, $F(1,250)=4.432$, $p<.05$) of the variance in DASS21 anxiety scores above and beyond the DASS21 anxiety variance accounted for by the covariates ($R^2=.048$, $F(4,251)=2.049$, $p<.05$). The consumption of poly-unsaturated fat was significantly associated with higher DASS21 anxiety scores $\beta = 3.802$, $t(250)=2.105$ $p<.05$.

For participants who completed the lab portion of the study, regression analysis were also run to determine the relationship between the self-reported scales (PSI, PSS, HADS-A, HADS-D, DASS21-A, DASS21-D and DASS21-S) from the survey data and the brief FNE, baseline positive affect and negative affect (PANAS) scales, and baseline physiology. Regression results indicated that consumption of saturated/mono-saturated fats accounted for 12.1% more of the variance in baseline negative affect (Change in R^2=.121, F(1,24)=5.072, p<.05), than was accounted for by the covariate predictors alone (R^2=.206, F(4,29)=1.878, p=.141). The consumption of saturated/mono-saturated fat was associated with less negative affect (β = 7.808, t(28)=-2.241, p<.05). Regression results also indicated that consumption of poly-unsaturated fats
accounted for 17.8% more variance in baseline positive affect (Change in R^2=.178, F(1,28??)=6.222, p<.05), than the covariate predictors alone (R^2=.022, F(4,29)=.165, p=.954). The consumption of poly-unsaturated fat was significantly associated with less positive affect β = -15.123, t(28)=-2.494 p<.05. Thus, these findings suggest that consumption of saturated/mono-saturated fat was associated with lower HADS depression and HADS negative affect, while consumption of poly-unsaturated fat was associated with higher DASS21 anxiety and lower DASS21 positive affect.

Consumption of poly-unsaturated fat was associated with poorer performance on the MIST. With regard to performance on the MIST, regression results predicting number of attempts on the initial MIST, indicate that consumption of poly-unsaturated fats accounted for 13.7% more of the variance (R^2=.396, F change(5,28)=6.365, p<.05) in attempts than the variance accounted for by the covariate predictors alone (Change in R^2=.259, F(4,29)=2.530, p=.062). The consumption of poly-unsaturated fat was

significantly associated with lower attempts on the initial MIST (β = -15.123, t(28)=-2.494 p=.05). Regression results predicting number of attempts on the second MIST, indicate that consumption of poly-unsaturated fats (Change in R^2=.138, F(5,28)=6.373, p<.05) predicted 13.8% more variance than was accounted for by the covariate predictors alone (R^2=.259, F change(4,29)=2.536, p=.061). The consumption of poly-unsaturated fat was significantly associated with lower attempts on the second MIST (β = -16.808, t(28)=-2.525 p=.05). Regression results predicting number of correct responses on the initial MIST, indicate that consumption of poly-unsaturated fats accounted for 12.2% more of the variance (Change in R^2=.122, F(5,28)=5.556, p<.05) than was accounted for by the covariate predictors alone (R^2=.265, F change(4,29)=2.613, p=.056). The consumption of poly-unsaturated fat was significantly associated with lower correct response on the initial MIST (β = -15.123, t(28)=-2.494 p=.05).

Laboratory Study

A 3 (group: high, moderate, and low fat consumption) by 5 (laboratory study phase: baseline, instruction, first MIST, second MIST, recovery) multivariate ANOVA was run on the physiological variables (SCL, HR, SV, CO, HF, and pnn50). Results revealed consistent phase effects across physiological variables (F(20,13)=7.748, Roy's GCR=12.913, p<.001), and no effect for group (p>.2) or group x phase interaction (p>.2). For the self-report measures, a 3 (group: high, moderate, and low fat consumption) by 2 (before first MIST, after second MIST) was run and consistent phase effects were found, indicating a change in affect in the direction of greater negativity following the second MIST for all participants. No other significant findings were revealed.

Baseline Values

A oneway multivariate ANOVA was run to analyze possible differences between fat consumption groups at baseline on self-report measures (carbohydrates, poly-unsaturated fat, saturated/mono-saturated fat, protein, PSI, PSS, HADS-A, HADS-D, DASS21-A, DASS21-D and DASS21-S, brief FNE and PANAS). There were no group differences in depression, anxiety, stress and somatic symptoms. There were also no differences in baseline values of positive and negative affect between the groups. With the exception of saturated/mono-saturated fat and protein consumption, no groups were different in any other baseline measures (table 3).

A oneway multivariate ANOVA was run to analyze possible differences between groups at baseline. Analysis revealed no statistically significant differences between fat consumption groups on any of the physiological variables ($F(12,54)=1.468$, $p>.2$; all univariate $ps>.1$).

Table 3
Self-Report Means and Standard Deviations

	Online N=256		Laboratory N=34					
			High Fat N=8		Moderate Fat N=17		Low Fat N=9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Carbs	0.48	0.123	0.39	0.139	0.49	0.111	0.4	0.15
Sat/Mono Fat	0.46	0.13	**0.62***	**0.12**	**0.47***	**0.04**	0.29	0.097
Poly Fat	0.49	0.183	0.5	0.188	0.42	0.197	0.29	0.197
Protein	0.58	0.184	**0.75***	**0.23**	0.48	0.192	0.34	0.29
Caffeine	1.05	0.919	1.25	1.164	1.88	2.42	1.22	0.833
PSI	21.12	5.424	21	7.8	22	5.894	19.22	5.974
PSS	15.34	6.159	14.5	9.53	13.76	6.118	17	6.264
HADS A	6.19	3.408	5.87	3.979	5.76	3.113	6.22	3.073
HADS D	2.85	2.561	2.87	3.044	2.58	2.501	3.44	2.554
DASS21 A	3.83	5.309	5.75	6.181	4.58	5.512	3.77	7.774
DASS21 D	4.62	5.867	5	6.502	4.11	4.029	2.66	4.472
DASS21 S	7.66	7.317	10.75	12.37	7.41	6.51	4.66	5.83
BFNE	NA	NA	28.25	10.646	34.75	10.148	29.22	11.177
PANAS P	NA	NA	27.25	7.186	28.11	7.532	31.11	6.03
PANAS N	NA	NA	11.12	0.834	13.05	3.782	12.55	3.004

Abbreviations: Carbs=Carbohydrates, Sat/Mono fat=Saturated/Mono-Saturated Fat, Poly Fat=Poly-Unsaturated Fat, PSI=Physical Symptoms Inventory, PSS=Perceived Stress Scale, HADS=Hospital Anxiety and Depression, DASS21=Depression, Anxiety and Stress Scale 21 item, BFNE=Brief Fear of Negative Evaluation, PANAS=Positive and Negative Affect Schedule Scale. **p<.05 Difference from low fat group*

Changes in Self-reported Affect

Repeated measures ANOVAs were run to determine changes in PANAS values from across the study phases. While there was no overall difference among groups in positive affect or negative affect, there was a significant increase in negative affect ($F(1,31)=8.385$, $p<.01$, $\eta^2=.213$) and decrease in positive affect ($F(1,31)=30.349$, $p<.001$, $\eta^2=.495$) following the stress tasks. All groups equally perceived the stress tasks as negative.

Changes in physiology

One between (fat consumption group) and one within (phase) repeated measures ANOVCAs with caffeine consumption as a covariate were run for all physiological measures (these represent follow-up analyses for the significant multivariate phase effect). For main effects, values were extracted from the Greenhouse-Geiser tests to correct for violations of the assumption of sphericity. Results indicated a significant effect of study phase on changes in SCL ($F(2.032, 60.950)=18.584$, $p<.001$, $\eta^2=.383$). Post-hoc analyses revealed significant increases in SCL from baseline (M=.8213, SD=1.875) to the instruction phase (M=2.662, SD=2.848; $p<.001$) the initial MIST (M=3.667, SD=3.411; $p<.001$), the second MIST (M=3.605, SD=3.769; $p<.001$) and recovery (M=2.495, SD=3.852; $p<.01$). Results for changes in HR also indicated a significant main effect for study phase $F(2.157, 64.697)=11.765$, $p<.01$, $\eta^2=.282$. Post hoc analysis revealed that HR significantly increased from baseline (M=78.392, SD=10.728) to the initial MIST (M=83.286, SD=10.428; $p<.001$) and the second MIST (M=84.286, SD=10.333; $p<.001$). There was no significant change in either CO or SV. The changes in SCL and HR indicate that all participants exerted effort during the three

task periods (instruction, initial mist and second MIST). With regard to heart rate variability, repeated measures ANOVA revealed a significant main effect for study phase on changes in pNN50 $F(3.119, 94.110)=3.119$, $p<.05$, $\eta^2=.094$.

Group Differnces

Simple effects for the repeated measures ANOVCA with caffeine consumption as a covariate, revealed within group differences with regard to the pNN50 and HF. For participants in the high fat group, pNN50 values decreased from baseline (M=26.458, SD=13.394) to the initial MIST (M=17.193, SD=12.566; p<.01) and the second MIST (M=17.952, SD=11.600; p<.05) (figure 3). Analyses also revealed that, during recovery, pNN50 (M=28.451, SD=13.589) was significantly higher than during the instruction phase (M=19.112, SD=11.398; p<.05), the initial MIST (M=17.193, SD=12.566; p=.001) and the second MIST (M=17.952, SD=11.600; p<.01). There were no significant changes in pNN50 for either the moderate fat group or the low fat group, although a simple comparison did reveal, for the low fat group, a significant decrease in pNN50 from the initial MIST (M=14.752, SD=13.584) to the second MIST (M=10.914, SD=10.849; p<.05) (figure 3).

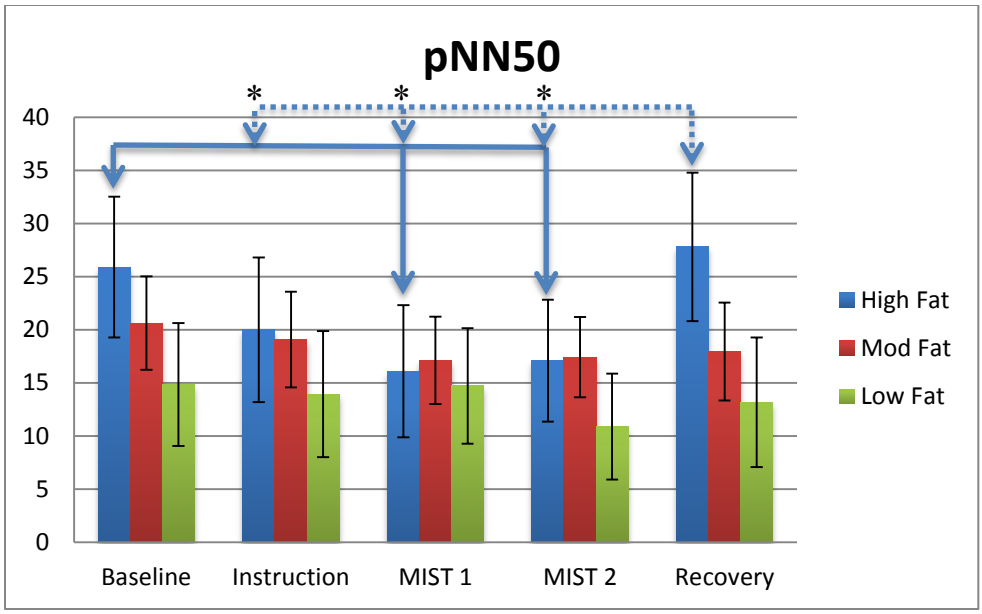


Figure 3 Results indicate that pNN50 values during baseline were significantly higher than MIST 1 and MIST 2. The recovery phase also indicated higher pNN50 values than instruction, MIST 1 and MIST2. Error bars represent 'standard error of the mean'. Significant differences (<.05) are marked with *.

For participants in the high fat group, HF during recovery (M=3.110, SD=.3787) was significantly higher than during the instruction phase (M=2.908, SD=.2752; p<.05), and the initial MIST (M=2.835, SD=.3038; p<.01) (figure 5). There were no significant changes in HF for the moderate fat or low fat group. Changes in pNN50 and HF indicate that the high fat group decreased from baseline levels and revealed a pattern similarly to the moderate fat and low fat groups during the three tasks periods. Following the tasks, participants in the high fat group recovered back to baseline levels, while the moderate and low fat group did not (figure 4).

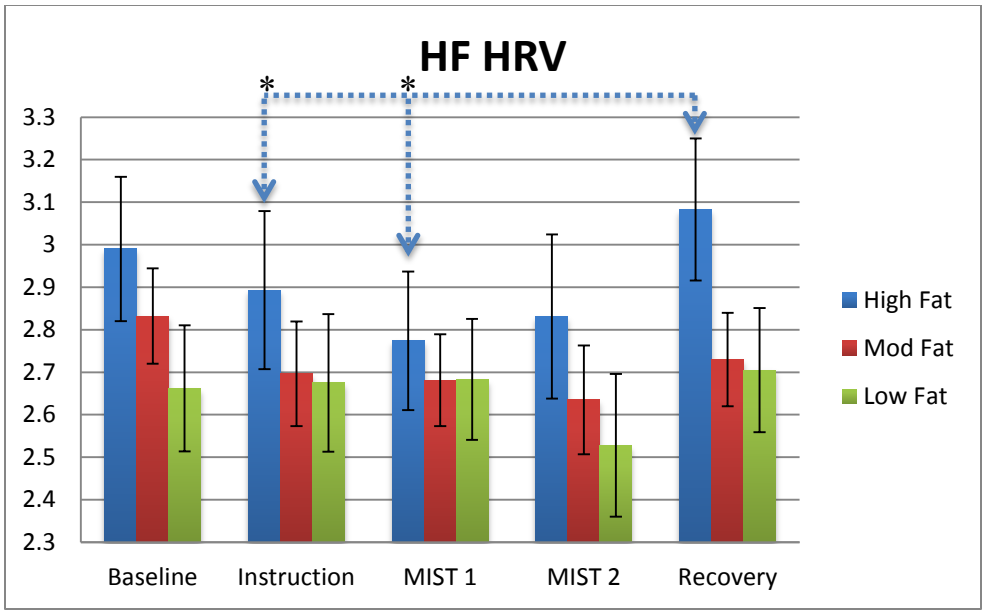


Figure 4 Results indicate that HF values during Baseline were significantly higher than MIST 2. The Recovery phase also indicated higher HF values than instruction, MIST 1 and MIST2. Error bars represent 'standard error of the mean'. Significant differences (<.05) are marked with *.

CHAPTER IV: DISCUSSION

The main purpose of the current study was to test whether the consumption of varying amounts of saturated/mono-saturated fats would provide any protection from anxiety, depression, stress and physical symptoms related to stress. The current study also sought to determine whether the consumption of saturated/mono-saturated fat would be associated with a more favorable autonomic response.

It was predicted that the high fat group would be associated with a more favorable autonomic response to the MIST task and this appears to be the case, though not exactly in the manner that was expected. It was hypothesized that the high fat group would be 'less reactive' to the MIST task than the moderate fat and low fat group, and that this would be evidenced by changes in pNN50 and HF. This prediction was based on the assumption that baseline values would not differ between groups and that the moderate fat and low fat groups would demonstrate *decreases* in HRV above and beyond that of

the high fat group. In this study, the high fat group proved to be 'more reactive' to the MIST and was the only group to demonstrate significant *increases* in pNN50 and HF values.

The changes in HRV in the high fat group may indicate better autonomic flexibility than either the moderate fat or low fat group. Participants' HRV in the moderate fat group did not vary much, and, the low fat group was the only group to demonstrate a significant *decrease* in HRV from the initial MIST to the second MIST task. Further, the low fat group showed little, if any, evidence of recovery following the MIST stressor. The high fat group on the other hand demonstrated flexibility. HRV significantly decreased in high fat participants, during the MIST, but significantly increased during recovery, returning high fat participants to their baseline. It seems that participants in the high fat group not only benefit from improved resting levels of HRV and enhanced recovery from an acute stress, but their data may also be taken as an indication of improved coping during acute stress.

Results from this study (unexpectedly) provided evidence that not all fats are equal. Participants consuming higher amounts of poly-unsaturated fat showed higher DASS21 anxiety scores and lower positive affect. The DASS21 anxiety scale was designed to indicate symptoms of anxiety related to hyperarousal and physical symptoms, therefore higher values on this scale may represent physical symptoms related to anxiety (Henry, & Crawford, 2005). Previous studies have also found that consumption of poly-unsaturated fat and trans fat to be anxiogenic, while consumption of saturated/mono-saturated fat was seen as both anxiolytic and anti-depressant (Murphy et al., 2013; Teixeira, et al., 2011).

Lower baseline values on the PANAS positive affect scale could also indicate a somewhat blunted ability to experience positive emotions, since the negative affect was not associated with poly-unsaturated fat consumption. These data may also suggest cognitive blunting associated with higher levels of poly-unsaturated fat. It was also found that higher reported consumption of poly-unsaturated fat was associated with fewer attempts on the initial MIST, the second MIST and it was also associated with lower correct attempts on the initial MIST. It is possible that consumption of higher levels of poly-unsaturated could be related to decreased effort and task performance. This would make sense since participants consuming more poly-unsaturated fat reported lower positive affect. Consumption of poly-unsaturated fat seems to be associated with a more negative state, while consumption of saturated/mono-saturated fat seems associated with a less negative state and more favorable autonomic response. Research will be needed to explore some of these relationships more fully.

The present study does not provide evidence for a specific mechanism for producing the autonomic effects related to increased consumption of saturated/mono-saturated fat in these data. This is especially true since there were no significant changes in levels of β-hydroxybutyrate observed between the groups. This is made more difficult because there is also no current consensus for how a ketogenic state may exert its benefits. Such difficulty has arisen when research has attempted to isolate any single element as the dominant mechanism accounting for the benefit provided by the ketogenic state (Forsythe et al., 2008; Foster et al. 2003; Masino et al., 2009; Politi et al., 2011 & Sato, et al., 1995). While serum levels of β-hydroxybutyrate from .5 up to 6 mmol indicates a mild ketogenic state and provides health benefits ranging from decreased

seizure frequency/severity to decreases in cardiovascular and diabetic risk factors, directly infusing β-hydroxybutyrate into subjects does not produce these same benefits. It may be that registering a high level β-hydroxybutyrate is possibly an artifact of being on the more extreme end of a high fat/low carbohydrate diet, while the consumption of greater amounts of saturated/mono-saturated fat may exert benefits along with a moderate intake of carbohydrates.

Participants in the high fat group demonstrated increased HRV, as well as enhanced HRV recovery following an acute stress, which may indicate increased parasympathetic influences over cardiovascular changes in this group. Buttressing the notion that the parasympathetic system may play an important role here, it has been found in other research that parasympathetic efferent communication with the liver is necessary to control the release of triglycerides into the blood stream, while sympathetic efferent activity to the liver produced negligible changes in serum triglyceride levels (BruinStroop, et al., 2013; Bruinstroop, et al., 2012). When parasympathetic efferent activity was severed, the amount of triglycerides released into the blood stream significantly increased. Increases in triglyceride levels have also been found to decrease the ability of leptin and to cross the blood brain barrier and exert its anxiolytic and anti-depressant effects (Considine, et al., 1996; Heymsfield, et al.,1999; Banks, DiPalma & Farrell, 1999). Multiple studies have found that high fat, low carbohydrate diets decrease serum levels of triglycerides and may also increase transport of leptin across the blood brain barrier (Forsythe et al., 2008; Foster et al., 2003; Volek et al., 2008; Volek et al., 2009; Volek et al., 2009). It may be that enhancement of parasympathetic activity is a

contributing mechanism for how a ketogenic diet lowers serum triglycerides, and this same mechanism may plausibly protect against the effects of acute stress.

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Appendix A: Informed Consent: Internet

IRB Protocol Number: 14.228
IRB Approval Date: February 25, 2014

University of Wisconsin – Milwaukee

Consent to Participate in Online Research

Study Title: Effect of Diet on Health and Cognition

Person Responsible for Research: Dr. Raymond Fleming, UWM Psychology Department & Shaun Stearns, Psychology Graduate Student

Study Description: The purpose of study is to examine the effects of diet on various aspects of health and cognition. This study will utilize dietary interventions to observe which one may be the most beneficial for improving a variety of health and cognitive measures. The initial survey portion of this study is designed to collect a variety of initial information relating to physiological and psychological health. The survey is also designed to screen out participants who would be a good fit for the lab portion of the study. Approximately 300 participants will participate in this portion of the study. If you agree to participate, you will be asked to complete a survey that will take approximately 60 minutes to complete. The questions will ask about physiological and psychological symptoms that you experience in your life, as well as questions about your health history and current eating habits. Data will be retained on the Qualtrics website server for 1 year and will be deleted after this time. However, data may exist on backups or server logs beyond the timeframe of this research project. Data transferred from the survey site will be saved in an encrypted format with a confidential ID number. Based on the information provided on this survey, some participants will be contacted to participate in the experimental portion of the study.

The second part of the study of the study is a 90 min laboratory session to measure cognitive ability and physiological measures. Not all willing participants will be selected and contacted. If selected to participate, a researcher will contact you by e-mail and assign you a code to sign up via SONA for the lab portion of the study. Including the 90 min lab portion, you will receive 2.5 hours total of lab credit.

Risks / Benefits: Risks to participants are considered minimal. You will be awarded 1 hour of research credit for the online survey portion and a potential of 1.5 more hours of research credit for the Lab portion. During the two laboratory portion, a finger stick will be performed to collect a single droplet of blood (i.e. blood glucose test); all other physiological measures will be recorded with adhesive electrodes. Extra credit for the participation in this study may not be guaranteed. Your instructor has final discretion for awarding extra credit.

Confidentiality: Every measure will be taken to keep your responses confidential. The internet site is public space. As an online participant in this research, there is always the risk of intrusion by outside agents (i.e., hacking) and therefore the possibility of being identified. To receive research credit and be contacted for further participation you will be asked to provide your name and phone number. Your course instructor will be given notice of your research participation. Data from this study will be saved on a password protected computer for up to ten years. Only Dr. Raymond Fleming and his research team will have access to the information. The only instance where your information will be disclosed is if there is suspected threat to self or others. In this instance, the appropriate authorities will be notified to ensure your or others safety.

Voluntary Participation: Your participation in this study is voluntary. You are free to not answer any questions and you may withdraw from this study at any time without penalty. Your decision will not change any present or future relationship with the University of Wisconsin-Milwaukee.

There are alternatives for extra credit other than participation in this study. Contact the UWM psychology department at 414-229-4746 or speak with your course instructor for possible alternatives.

Who do I contact for questions about the study: For more information about the study or study procedures, contact Dr. Raymond Fleming at 414-229-3980 (or mundo@uwm.edu).

Who do I contact for questions about my rights or complaints towards my treatment as a research subject? Contact the UWM IRB at 414-229-3173 or (irbinfo@uwm.edu).

Research Subject's Consent to Participate in Research:

To voluntarily agree to take part in this study, you must complete the attached survey. Completing the survey indicates that you have read this consent form and have had all of your questions answered.

Thank you!

CONSENT 1 - Do you agree to the consent information listed on this form?

- Yes, I agree to the above consent form.
- No, I don't agree to the above consent form.

CONSENT 2 - Are you 18 years or older?
IF YOU ARE NOT 18 YOU ARE NOT ELIGIBLE TO PARTICIPATE.

- Yes, I am over 18.
- No, I am under 18.

Appendix B: Informed Consent: Laboratory

UNIVERSITY OF WISCONSIN – MILWAUKEE
CONSENT TO PARTICIPATE IN RESEARCH

THIS CONSENT FORM HAS BEEN APPROVED BY THE IRB FOR A ONE YEAR PERIOD

1. General Information

Study title:
- Effect of Diet on Health and Cognition

Person in Charge of Study (Principal Investigator):
- Raymond Fleming, PhD
- Faculty Member, Psychology Department

2. Study Description

You are being asked to participate in a research study. Your participation is completely voluntary. You do not have to participate if you do not want to.

Study description:
The purpose of this study is to
- Understand the effects of Diet on Cognition.
- To determine what aspects of diet are most beneficial to cognition.
- Cognitive tasks will be performed in lab Pearse B76.
- There will be 60 subjects total participating in this portion of the study.
- The duration of the study will be a 90 min lab session.

3. Study Procedures

What will I be asked to do if I participate in the study?

- Perform a math based cognitive task known as the MIST, which is a computerized arithmetic task. It will require a 5-minute resting baseline, 5-minute orienting task, 5-minute experimental task and 5-minute recovery period.

- Complete the brief FNE. The brief fear of negative evaluation questionnaire asks a variety of questions related to your possible fear of negative social evaluation.

- Complete a pre and post task PANAS. The Positive and Negative Affective Schedule provides a series emotion related adjectives and you will indicate how much you are feeling each one.

A variety of physiological measures will be recorded during the cognitive task portion of the study.

- A droplet of blood will be collected from a finger stick to measure blood ketone levels.

- There will be adhesive electrodes connected to your neck, lower back, left hand, left ankle and under your right color bone. These electrodes are necessary to collect impedance cardiography, electrocardiography and skin conductance measures.

The lab portion of the task will require 90 minutes.

4. Risks and Minimizing Risks

What risks will I face by participating in this study?

- Psychological risk:
  - Distress caused by the cognitive task (less likely)
    - The Norris Health center will be available if any distress is caused by the cognitive task
- Physical Risks
  - A finger stick will be used to draw a droplet of blood (e.g. blood glucose meter)
    - This procedure is relatively painless and over in a second
- Online Survey Risks
  - Every measure will be taken to keep your responses confidential. Though the internet site is a public space. There is always the risk of intrusion by outside agents (i.e., hacking) and therefore the possibility of being identified. Data will be stored on secure servers and password protected computers.

5. Benefits

Will I receive any benefit from my participation in this study?

- The results from this study will also help to further elaborate the connection between diet and cognition.

6. Study Costs and Compensation

Will I be charged anything for participating in this study?

- You will not be charged anything.

Are you paid or given anything for being in the study?

- Completion of the initial survey will provide 1 hour of lab credit.
- Completion of the lab portion will provide an additional 1.5 hours of lab credit.
    - The total lab credit received would be 2.5 hours to be split into 1 hour for 1 class and 1.5 hours for another class (or the same class).

Are there alternatives to participating in this study?

- There are other studies available through the SONA which will also provide lab credit

7. Confidentiality

What happens to the information collected?

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. We may decide to present what we find to others, or publish our results in scientific journals or at scientific conferences. Only the PI will have access to the information. However, the Institutional Review Board at UW-Milwaukee or appropriate federal agencies like the Office for Human Research Protections may review this study's records.

- Participant information will be coded to protect the identity of participants.
- All information will be stored in a locked office on a password protected computer.
- The data will be stored in Pearse B80 for 1 year for future use.
- Identifiable electronic data will be stored on secure password protected Qualtrics servers.

8. Alternatives

- You have the option of participating in other research on campus. These studies are available through SONA and will also provide lab credit.

9. Voluntary Participation and Withdrawal

What happens if I decide not to be in this study?

Your participation in this study is entirely voluntary. You may choose not to take part in this study. If you decide to take part, you can change your mind later and withdraw from the study. You are free to not answer any questions or withdraw at any time. Your decision will not change any present or future relationships with the University of Wisconsin Milwaukee.

- Refusal or withdrawal from the study will not impact your grade or class standing.

10. Questions

Who do I contact for questions about this study?
For more information about the study or the study procedures or treatments, or to withdraw from the study, contact:

Raymond Fleming
University of Wisconsin-Milwaukee
228 Garland Hall
2441 E. Hartford Ave.
Milwaukee, WI 53211
(414) 229-3980

Who do I contact for questions about my rights or complaints towards my treatment as a research subject?
The Institutional Review Board may ask your name, but all complaints are kept in confidence.

Institutional Review Board
Human Research Protection Program
Department of University Safety and Assurances
University of Wisconsin – Milwaukee
P.O. Box 413
Milwaukee, WI 53201
(414) 229-3173

11. Signatures

Research Subject's Consent to Participate in Research:
To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study, you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read or had read to you this entire consent form, including the risks and benefits, and have had all of your questions answered, and that you are 18 years of age or older.

_____
Printed Name of Subject/ Legally Authorized Representative

_____ _____
Signature of Subject/Legally Authorized Representative Date

Principal Investigator (or Designee)
I have given this research subject information on the study that is accurate and sufficient for the subject to fully understand the nature, risks and benefits of the study.

_____ _____
Printed Name of Person Obtaining Consent Study Role

_____ _____
Signature of Person Obtaining Consent Date

Appendix C: Researcher Script

Diet and Health Lab Script **(Read out loud the parts in BOLD)**

Set up:
-Make sure electrodes are ready.
-Prepare ketone meter, ketone strip, lancet, cotton balls, alcohol, latex gloves
-Make sure stopwatch, measuring tape and forms are accessible and ready for use.

Computer:
-Open excel sheet for calculating LVET
-Open excel sheet for participant coding.
-Open "Impedance Template" file and save as "Participant number" in "Diet and Cognition" folder.

Forms: (2 Informed consent and worksheet)
-Write down incoming "participant number" on Worksheet and Debriefing form.

Begin Script

Hello! Welcome to the diet and cognition study!

Close door behind participant.

Please have a seat at the desk.

Hand the participant 2 informed consent forms.

First off, please look over the informed consent. If everything is in order and you agree to participate, please provide your signature at the end of this form. The second copy is for you to keep.

Just to check, could you please tell me your full name? Type name into Excel coding, then close.

Before we get begin could you please turn off your cell phone. Make sure yours is off as well.

All right! Let's get started.

You will now complete 2 short surveys, stop after the second one. These questions are designed to measure how you feel right now, before doing the cognitive task. If you have any questions or you've completed the surveys, please let me know by raising your hand.

Bring up "Web Browser" and click on "In Lab Questionnaire" bookmark, type in participant number and then leave the room until they are finished.

Minimize Firefox once they are done.

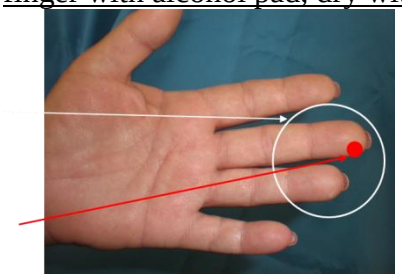
Thank you!

We will now need to get a drop of blood from you in order to test your blood level of ketones. (Prepare ketone meter and put on latex gloves)

Would you prefer to use the lancet or would you like me to operate it? If you could, please massage your hand like this 5 times (demonstrate).

If they choose to do it, instruct them where on their hand to use it. (Clean MIDDLE finger with alcohol pad, dry with cotton ball)

If they prefer that you do it, count to 3 and activate the lancet. (Clean finger with alcohol pad, dry with cotton ball)



I will now massage your finger to squeeze out a larger droplet. (Squeeze upwards on the participants finger).

Touch ketone strip to blood drop, write down the value.

Use dry cotton ball to clean the blood spot. Provide clean cotton ball and have participant apply pressure to stick site. Throw the lancet and used ketone strip away.

Thank you! Please hold this cotton ball until I've connected the electrodes on your neck.

We will now attach electrodes to record physiological measures and start the cognitive task. Look towards file cabinet for illustration.

ICG: Attach neck electrodes while participant is seated; have them stand to attach the back electrodes. (Measure distance from bottom neck electrode to top back electrode. Write down in centimeters). Have the participant sit down and hold the ICG wires so she does not sit on them. Present trash can so the participant can dispose of the cotton ball.

ECG: Attach Leads to electrodes and then pre-gel the electrodes.

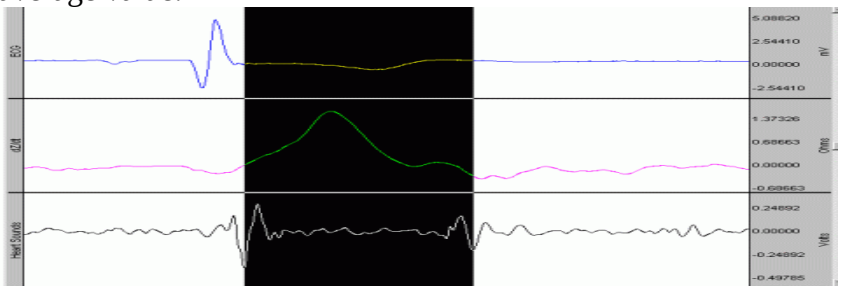
Please attach this cable under your right collarbone. Hand them the white electrode. **Please attach this electrode on the inside of your left ankle.** Hand them the red electrode.

Bring up the BSL file, click on "MP35" and set "acquisition" for 30 seconds (click reset).

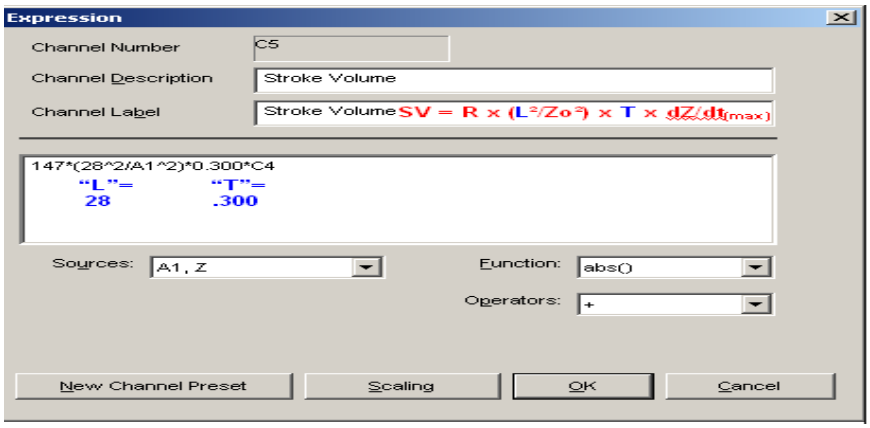
First we will need to record for a short period in order to calibrate the physiological measurements. Try to get as comfortable as possible, keep your feet flat and your left hand on your leg to eliminate noise from the recording. Put black cloth tarp over screen and start recording.

I will now be calibrating the physiological recordings; this will only take a minute or two.

Measure LVET from 10 clean heartbeats. Click scale, change to 1.0 and click OK. Right click "copy to clipboard" and paste data to excel (ctrl-V) and write down average value.



Insert values into Cardiac Output calculation. Distance between strips (L) and average LVET (T).



We will now record a 5-minute rest period. Make yourself as comfortable as possible, keeping your feet on the ground and your left hand on your leg to eliminate noise from the recording. I will be in the middle room and will be back in 5-minutes. Please raise your hand if you need assistance.

Set acquisition for 5-minutes (DO NOT CLICK RESET), hit start on Biopac and stopwatch simultaneously. Leave the room.

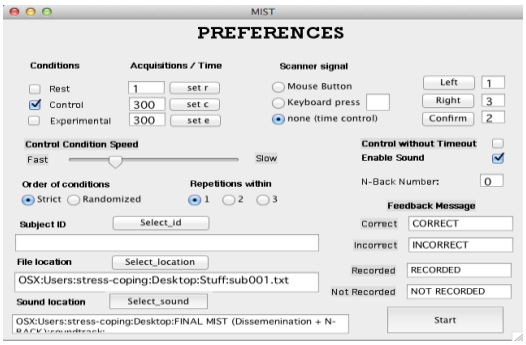
Remove black cloth tarp from screen

What you are now about to perform is a math based cognitive task.

(Present a picture of the MIST interface while explaining)

The rotary dial can be moved left and right in order to select an answer. The number 1 on the numeric pad moves the dial to the left, number 3 moves the dial to the right and number 2 selects the answer. You will have a limited amount of time to answer each question. You will now begin a five-minute session so you can get used to the task. Try to get as comfortable as possible, keep your feet flat and your left hand on your leg to eliminate noise from the recording. Please raise your hand if you need assistance.

Set up MIST. Start the MIST and Biopac simultaneously. Start stopwatch and leave room for 5 minutes.



Once done: Write down amount correct and incorrect.

(Present a picture of the MIST interface while explaining again)

<u>We will now begin the actual cognitive task.</u> During this task you will hear a timing bar count down during each question and receive real time feedback about your level of performance. <u>The bar above the math question indicates how you are performing in comparison to the average of a large pilot sample.</u>

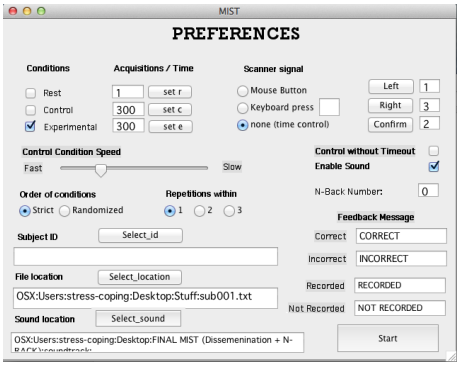
<u>The red zone is below average, the yellow zone is average and the green zone is above average. You need to stay in the yellow zone and try to get into the green zone. If you go too far into the red zone your data may not be useable and you may have to perform the task again.</u>

I will be observing during this condition.

Try to get as comfortable as possible, keep your feet flat and your left hand on your leg to eliminate noise from the recording.

Set up MIST. Start the MIST and Biopac simultaneously, stay in room where the tape is marked.

<u>-IMPORTANT- for or Sound</u>**<u>:</u>** Click on "Select Sound", click "Final Mist", then click "Soundtrack" and click OK. This must be done or there will be no sound. Simultaneously hit start on Biopac and the MIST.



Once done: Write down amount correct and incorrect.

Important! Show subtle disappointment. **Okay…**(long pause) **We are going to have to try this one more time and let's see if you can perform a LITTLE better…**(long pause). **This time I want you to do everything in your power to beat your previous attempt.**

I will be observing this condition as well.

Try to get as comfortable as possible, keep your feet flat and your left hand on your leg to eliminate noise from the recording.

Set up MIST. Start the MIST and Biopac simultaneously, stay in room where the tape is marked.

Once done: Write down amount correct and incorrect.

All right, you have now completed the cognitive task. I will now record a 5-minute recovery period. Make yourself as comfortable as possible, keeping your feet on the ground and your left hand on your leg to eliminate noise from the recording. I will be in the middle room and will be back in 5-minutes. Please raise your hand if you need assistance.

Place black cloth tarp over screen.

Hit start on Biopac and stopwatch simultaneously. Leave the room.

I will now remove your adhesive electrodes.

Remove electrodes from participant

The following surveys are interested in how you currently feel after completing the cognitive task. Please scroll down as you complete the survey and if you have any questions or you've completed the surveys, please let me know by raising your hand.

Bring "firefox" back up. Continue onto the next screen and have the participant complete the rest of the questionnaires.

Leave room.

I will now need you to complete a food journal indicating what you have eaten from 3 days ago up till today. If you have any questions or you've completed the food journal, please let me know by raising your hand.

Open Web Browser and click on “Food Journal” bookmark. Then leave room.

Close Web Browser once they are done.

I will now do a short debriefing interview, just to ask some basic questions.

Use debriefing from to ask questions.

What you have just performed is known as the “Montreal imaging stress task” or the MIST. The task was designed to be challenging and elicit a stress response from you. The feedback you received was part of the manipulation in order to increase your effort on the task. The goal of the task was to maximize the amount of effort you put into it. You performed very well! Thanks you so much for participating! You may now gather your belongings.

*****Save Biopac file*****

Appendix D: Demographics, Health and Diet Questionnaire

Demographic information

1. Please clearly fill in the requested contact information below. We need this information so that we can contact you if you are selected to participate in related research or to arrange credit for your participation. So please supply contact information that is best for you. All information you provide throughout this survey will be kept confidentioal.

   First Name_____

   Last Name_____

   Phone_____

   Email_____

2. Your gender is:

   Male_____ Female_____

3. How old are you?

   _____years old

4. What is your height?

   _____foot/inches

5. Are you:

   Married_____

   Divorced_____

   Widowed_____

   Never married_____

6. What is the highest level or degree of school that you have completed?

   - Some college credit, but less than 1 year____
   - 1 or more years of college, no degree____
   - Associate degree (for example, AA, AS)____
   - Bachelor's degree (for example, BA, AB, BS)_____

5. Are you employed?

   Yes_____ No_____

6. Your ethnicity is:

   - Caucasian_____

- African American_____
- Asian American_____
- Hispanic_____
- Other (please, indicate your ethnicity)_____

Cardiovascular Information

1. Do you have a history of any of the following cardiovascular problems:
    a. Hypertension (high blood pressure)
    b. Coronary Artery Disease
    c. Atherosclerosis (hardening of the arteries)
    d. Stroke
    e. Myocardial Infarction (heart attack)
    f. Aortic stenosis (decreased blood flow from the heart)
    g. Mitral regurgitate
    h. Any other cardiovascular disease not listed above (please, indicate the name of this disease)_____
    i. I DO NOT HAVE ANY CARDIOVASCULAR PROBLEMS

2. Do you have any respiratory problems?
    a. Yes
    b. No
3. If you answered yes, please indicate what type of respiratory problem you have. _____

4. Have you been diagnosed with Major Depressive Disorder and/or Generalized Anxiety Disorder?
    a. Yes
    b. No

5.Do you currently take medication in order to treat symptoms of depression and/or anxiety?
    a. Yes

b. No

6. Do you currently take any of the following medications in any form:
    a. Dexamethasone
    b. Steroids (e.g., prednisone, or inhaled steroids for asthma)
    c. Diet pills (please, indicate the name of the pill:_____)
    d. Beta-blockers
    e. Anti-histamines
    f. Decongestants
    g. Any other medications not listed above (please, write the name of this medication and what it is treating)_____
    h. I DO NOT CURRENTLY TAKE ANY MEDICATIONS
5. Do you smoke?
    a. Yes
    b. No
6. If you smoke, how many cigarettes do you smoke per day?_____
7. How much caffeine/caffeinated beverages do you USUALLY consume per day?
    a. How many 8 oz. cups of coffee and/or tea do you have per day?_____
    b. Please, list other caffeinated beverages/foods you may have during the day_____
8. How many times a week do you exercise?
    a. Less than once a week
    b. Once a week
    c. Twice a week
    d. Three times a week
    e. Four or more times a week
9. How vigorous is your exercise?
    a. Very intense (such as fast jogging, weight lifting, etc.)

b. Moderate (such as slow jogging, fast walk)
c. Light (such as walking to school)

10. Do you have an aversion to any of the following dietary choices?
    a. Veganism (no meat or animal byproducts)
    b. Vegetarianism (no meat, but eat milk, eggs, etc.)
    c. Low Carbohydrate/Paleo (mostly fat, moderate protein and vegetables)
    d. I have no aversion to any of these choices
11. Are you currently eating according to any of the following dietary choices?
    a. Veganism (no meat or animal byproducts)
    b. Vegetarianism (no meat, but eat milk, eggs, etc.)
    c. Low Carbohydrate/Paleo (mostly fat, moderate protein and vegetables)
    d. Other_____
12. How often do you consume… RARELY **1 2 3 4 5** OFTEN
    a. Bread_____
    b. Soda (caffeinated or not) _____
    c. Hard Candy_____
    d. Chocolate_____
    e. Sports Drinks_____
    f. Fruit Juice_____
    g. Whole Fruit_____
    h. Potatoes_____
    i. Rice_____
    j. Cereal_____
    k. Bagels_____
    l. Sandwiches_____
    m. Chips (potato, corn and/or wheat) _____
    n. Low fat dairy_____
    o. Processed Meats (deli meat, cured bacon, jerky, cured sausages) _____
    p. Full fat dairy_____
    q. Butter_____

r. Cheese_____
s. Cream cheese/Sour cream_____
t. Olive oil_____
u. Vegetable Oils (Corn, Soy, Peanut, Canola, safflower, Pam)_____
v. Coconut oil_____
w. Pork_____
x. Chicken_____
y. Steak_____
z. Fish_____

Appendix E: Brief Fear of Negative Evaluation

This questionnaire is concerned with thoughts or feelings people may have in social situations. Please read each item carefully and rate the degree to which each item is characteristic of you on a scale from 1 (not at all characteristic of me) to 5 (extremely characteristic of me).

1. I worry about what people will think of me even when I know it doesn't make any difference.	1	2	3	4	5
2. I am unconcerned even if I know people are forming on unfavorable impression of me.	1	2	3	4	5
3. I am frequently afraid of other people noting my shortcomings.	1	2	3	4	5
4. I rarely worry about what kind of impression I am making on someone.	1	2	3	4	5
5. I am afraid that others will not approve of me.	1	2	3	4	5
6. I am afraid that people will find fault with me.	1	2	3	4	5
7. Other people’s opinions of me do not bother me.	1	2	3	4	5
8. When I am talking to someone, I worry about what they may be thinking about me.	1	2	3	4	5
9. I am usually worried about what kind of impression I make.	1	2	3	4	5
10. If I know someone is judging me, it has little effect on me.	1	2	3	4	5
11. Sometimes I think I am too concerned with what other people think of me.	1	2	3	4	5
12. I often worry that I will say or do the wrong things.	1	2	3	4	5

Appendix F: Positive and Negative Affective Scale

PANAS Questionnaire

This scale consists of a number of words that describe different feelings and emotions. Read each item and then list the number from the scale below next to each word. **Indicate to what extent you feel this way right now, that is, at the present moment.**

1	2	3	4	5
Very Slightly or Not at All	A Little	Moderately	Quite a Bit	Extremely

_____ 1. Interested	_____ 11. Interested
_____ 2. Distressed	_____ 12. Distressed
_____ 3. Excited	_____ 13. Excited
_____ 4. Upset	_____ 14. Upset
_____ 5. Strong	_____ 15. Strong
_____ 6. Guilty	_____ 16. Guilty
_____ 7. Scared	_____ 17. Scared
_____ 8. Hostile	_____ 18. Hostile
_____ 9. Enthusiastic	_____ 19. Enthusiastic
_____ 10. Proud	_____ 20. Proud

Appendix G: Three Day Food Journal

Please list the foods and beverages you consumed 3 days ago, 2 days ago, yesterday and today. Do not feel limited by the boxes, they will expand as you type. If you skipped a meal, then leave the box blank and move onto the next item.

3 Days ago

Breakfast

Lunch

Dinner

Snacks (e.g. chips, candy and/or chocolate)

Beverages

8 oz cups of water

8 oz cups of coffee, tea and/or energy drink

Cans of soda

Bottles of soda

8 oz cups of Fruit Juice

Sports Drinks

Total

2 Days ago

Breakfast

Lunch

Dinner

Snacks (e.g. chips, candy and/or chocolate)

Beverages

8 oz cups of water

8 oz cups of coffee, tea and/or energy drink

Cans of soda

Bottles of soda

8 oz cups of Fruit Juice

Sports Drinks

Total

1 Day ago

Breakfast

Lunch

Dinner

Snacks (e.g. chips, candy and/or chocolate)

Beverages

8 oz cups of water

8 oz cups of coffee, tea and/or energy drink

Cans of soda

Bottles of soda

8 oz cups of Fruit Juice

Sports Drinks

Total

Today. If you haven't eaten a meal yet, just leave blank and skip to the next item.

Breakfast

Lunch

Dinner

Snacks (e.g. chips, candy and/or chocolate)

Beverages

8 oz cups of water

8 oz cups of coffee, tea and/or energy drink

Cans of soda

Bottles of soda

8 oz cups of Fruit Juice

Sports Drinks

Total

Appendix H: Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? 0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life? 0 1 2 3 4

3. In the last month, how often have you felt nervous and stressed? 0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems? 0 1 2 3 4

5. In the last month, how often have you felt that things were going your way? 0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things you had to do? 0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life? 0 1 2 3 4

8. In the last month, how often have you been angered because of things that were outside of your control? 0 1 2 3 4

10. In the last month, how often have you felt difficulties were pilling up so high that you could not overcome them? 0 1 2 3 4

Appendix I: Physical Symptoms Index

Over the past month, how often have you experienced each of the following symptoms?	**Not at all**	**Once or Twice**	**Once or twice per week**	**Most days**	**Every day**
1. An upset stomach or nausea	1	2	3	4	5
2. Trouble sleeping	1	2	3	4	5
3. Headache	1	2	3	4	5
4. Acid indigestion or heartburn	1	2	3	4	5
5. Eye strain	1	2	3	4	5
6. Diarrhea	1	2	3	4	5
7. Stomach cramps (Not menstrual)	1	2	3	4	5
8. Constipation	1	2	3	4	5
9. Ringing in the ears	1	2	3	4	5
10. Loss of appetite	1	2	3	4	5
11. Dizziness	1	2	3	4	5
12. Tiredness or fatigue	1	2	3	4	5

Appendix J: Hospital Anxiety and Depression Scale

Hospital Anxiety and Depression Scale (HADS)

A	**I feel tense or 'wound up':**	
	Most of the time	3
	A lot of the time	2
	From time to time, occasionally	1
	Not at all	0

D	**I still enjoy the things I used to enjoy:**	
	Definitely as much	0
	Not quite so much	1
	Only a little	2
	Hardly at all	3

A	**I get a sort of frightened feeling as if something awful is about to happen:**	
	Very definitely and quite badly	3
	Yes, but not too badly	2
	A little, but it doesn't worry me	1
	Not at all	0

D	**I can laugh and see the funny side of things:**	
	As much as I always could	0
	Not quite so much now	1
	Definitely not so much now	2
	Not at all	3

A	**Worrying thoughts go through my mind:**	
	A great deal of the time	3
	A lot of the time	2
	From time to time, but not too often	1
	Only occasionally	0

D	**I feel cheerful:**	
	Not at all	3
	Not often	2
	Sometimes	1
	Most of the time	0

A	**I can sit at ease and feel relaxed:**	
	Definitely	0
	Usually	1
	Not Often	2
	Not at all	3

D	**I feel as if I am slowed down:**	
	Nearly all the time	3
	Very often	2
	Sometimes	1
	Not at all	0

A	**I get a sort of frightened feeling like 'butterflies' in the stomach:**	
	Not at all	0
	Occasionally	1
	Quite Often	2
	Very Often	3

D	**I have lost interest in my appearance:**	
	Definitely	3
	I don't take as much care as I should	2
	I may not take quite as much care	1
	I take just as much care as ever	0

A	**I feel restless as I have to be on the move:**	
	Very much indeed	3

	Quite a lot	2
	Not very much	1
	Not at all	0

D	**I look forward with enjoyment to things:**	
	As much as I ever did	0
	Rather less than I used to	1
	Definitely less than I used to	2
	Hardly at all	3

A	**I get sudden feelings of panic:**	
	Very often indeed	3
	Quite often	2
	Not very often	1
	Not at all	0

D	**I can enjoy a good book or radio or TV program:**	
	Often	0
	Sometimes	1
	Not often	2
	Very seldom	3

	Scoring (add the As = Anxiety. Add the Ds = Depression). The norms below will give you an idea of the level of Anxiety and Depression.	
	0-7 = Normal	
	8-10 = Borderline abnormal	
	11-21 = Abnormal	

Appendix K: Depression, Anxiety and Stress Scale

DASS 21

Please read each statement and circle a number 0, 1, 2 or 3 that indicates how much the statement applied to you *over the past week.* There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all

1 Applied to me to some degree, or some of the time

2 Applied to me to a considerable degree, or a good part of time

3 Applied to me very much, or most of the time

1S	I found it hard to wind down	0	1	2	3
2A	I was aware of dryness of my mouth	0	1	2	3
3D	I couldn't seem to experience any positive feeling at all	0	1	2	3
4A	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5D	I found it difficult to work up the initiative to do things	0	1	2	3
6S	I tended to over-react to situations	0	1	2	3
7A	I experienced trembling (eg, in the hands)	0	1	2	3
8S	I felt that I was using a lot of nervous energy	0	1	2	3
9A	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10D	I felt that I had nothing to look forward to	0	1	2	3

11S	I found myself getting agitated	0	1	2	3
12S	I found it difficult to relax	0	1	2	3
13D	I felt down-hearted and blue	0	1	2	3
14S	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15A	I felt I was close to panic	0	1	2	3
16D	I was unable to become enthusiastic about anything	0	1	2	3
17D	I felt I wasn't worth much as a person	0	1	2	3
18S	I felt that I was rather touchy	0	1	2	3
19A	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20A	I felt scared without any good reason	0	1	2	3
21D	I felt that life was meaningless	0	1	2	3

D=Depression

A=Anxiety

S=Stress

Apply template to both sides of sheet and sum scores for each scale.

For short (21-item) version, multiply sum by 2.

Appendix L: Debriefing

The debriefing will begin with a short open conversation about the participant's experience and perception of the study. Then the following questions will be asked.

Do you currently consider yourself to be eating a healthy diet? Yes or No

What would you consider a healthy diet?

What foods do you think you should be eating more of?

What foods do you think you should be eating less of?

Would you consider Saturated Fat to be healthy (coconut oil, animal fats)?Yes or No

Would you consider Mono-Saturated fat to be healthy (olive oil)? Yes or No

Would you consider Poly-Unsaturated Fats to be healthy (vegetable oils)? Yes or No

What foods do you feel could be consumed in high amounts and still be considered healthy?

What did you perceive the purpose of the MIST computer task to be?

Do you have any questions or comments you would like to bring our attention to?

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Curriculum Vitae
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EDUCATION

University of Wisconsin-Milwaukee **Milwaukee, Wisconsin**

Graduate:
University of Wisconsin-Milwaukee
Program: Health Psychology-PhD Program
Degree: MA Health Psychology
Attending: 2007-Present
Research: "Physiological Benefits of Cardiovascular Entrainment on Emotion Regulation"
Advisor: Raymond Fleming PhD

Undergraduate:
Shepherd University, Shepherdstown, West Virginia
Program: General Psychology
Degree: B.A.- Psychology, Minor-Literature

RESEARCH EXPERIENCE

University of Wisconsin-Milwaukee **Milwaukee, Wisconsin**
Department of Psychology

Principal Investigator **September, 2008 – May, 2010**

- Masters Thesis: "Diminishing the Stress Response: The Effect of Entrainment on the Physiological Response to and Perceived Severity of Negative Stimuli"
- Conducting an empirical study on effects of manipulating simple hand movements and the subsequent change in physiological arousal and emotions (positive/negative).
    - **Physiological Measures**: Electrocardiogram, Electrodermal activity, Respiration, Peak-to-Valley Respiratory Sinus Arrhythmia,
    - **Self-Report Measures:** Positive and Negative Affective Scale, Self-Assessment Manikin

Committee Members: Raymond Fleming, Ph.D., Diane M. Reddy Ph.D. & Shawn Cahill, Ph.D.

Research Assistant **January, 2012 – May 2013**

- U-pace online learning environment
- Data Analysis and Processing
- Award winning model for higher education
    - 2012 Distance Education Innovation Award (National Technology Network)
    - 2013 Desire2EXCEL Impact Award

Principal Investigators: Diane Reddy, PhD. & Raymond Fleming, Ph.D.

Principal Investigator **September, 2014 – Present**

- Doctoral Dissertation: "Effects of a Low Carbohydrate or Low Fat Diet on Coping with Acute Stress"
    - **Physiological Measures:** Impedance Cardiography, Electrocardiogram, Stroke Volume, Cardiac Output, Electrodermal Activity and Heart Rate Variability
    - **Self-Report Measures:** Hospital Anxiety and Depression Scale, Depression Anxiety and Stress Scale, Positive and Negative Affect Scale, Mini International Neuropsychiatric Interview 600, Perceived Stress Scale

Committee Members: Raymond Fleming, Ph.D., Diane M. Reddy Ph.D., Han Joo Lee, Ph.D., Christine Larson, Ph.D., & Hobart Davies, Ph.D.

TEACHING EXPERIENCE

University of Wisconsin-Milwaukee **Milwaukee, Wisconsin**

2007 Teaching Assistant, University of Wisconsin-Milwaukee
- Social Psychology

2007-09 Teaching Assistant, University of Wisconsin-Milwaukee
- Introduction to Psychology

2009-14 Teaching Assistant, University of Wisconsin-Milwaukee
- Psychophysiology

2010-11 **Associate Lecturer**
- Introduction to Psychology

2011-12 **Associate Lecturer**
- Introduction to Psychology

University of Wisconsin-Parkside **Kenosha, Wisconsin**

2012-13 **Associate Lecturer**
- Research Methods

HONORS

2011 **Certificate: "**Person named by first-year students who helped them the most in their college success" UWM Student Success Center

PRESENTATIONS

2014 **Proposed Doctoral Dissertation** January 10, 2014

2013 **Poster Presentation:** "U-Pace: A new Model for online instruction in higher education", Annual Georgia Conference on College and University Teaching.

2013 **Poster Presentation:** "Learning How to Learn: U-Pace Instruction Facilitates Study Skill Development", American Psychological Association.

2012	**Poster Presentation:** "The Effects of Manipulating Repetitive Action on Cardiovascular Activity", Association for Psychological Science.
2011	**Poster Presentation:** "Decreasing arousal through manipulation of repetitive action", Midwestern Psychological Association.
2011	**Invited Speaker:** National Kidney Foundation of Wisconsin "Introduction to the Methodology of Tai Chi" February 17, 2011.
2010	Poster Presentation: "Diminishing the Stress Response: The Effect of Entrainment on the Physiological Response to and Perceived Severity of Negative Images", Midwestern Psychological Association (April 30th, 2010).
2010	Successfully Defended Masters Thesis, March 9, 2010
2009	Poster Presentation: "Entrainment of Heart Rate Variability Through Finger Tapping", Midwestern Psychological Association.
2007-09	APAGS Representative: Promoted advancement and political action for topics relating to APA, as well as the "Health Parity Act" passed through state legislature and signed into law.
2006	Poster Presentation: "The Effects of Uncontrollable Stress on Perceived Self-Efficacy", Mid-Atlantic Undergraduate Social Research Conference.

PUBLICATIONS

2014	Stearns, S. S., Fleming, R., & Fero, L. J. (2014). Regulating emotional arousal through the manipulation of simple hand movements. *Psychophysiology.* Manuscript submitted for publication.

REFERENCES

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