

Effect of Hydrothermodynamic (HTD)-Processed Blueberries on Postprandial Blood  
Glucose Control and Antioxidant Status in Human Adults

By

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The prevalence of diet-related chronic diseases continues to rise in Canada and globally. Healthy lifestyle factors including healthy diets and physical activity are sustainable means of reducing risks of developing chronic diseases. Adequate intake of fruits provides health benefits due to their fiber content and polyphenols. However, conventional processing methods may negatively affect these benefits. Hydrothermodynamic technology (HTD) is a method employing the principle of Venturi cavitation that leads to collision of particles in the turbulent fluid and is used for processing of fruits and berries into puréed products. The aim of this study was to determine the effect of HTD-processed blueberries on postprandial blood glucose control and antioxidant status in human adults. Methods: 25 healthy adults 19-35 years old participated in the study. Using a randomized controlled cross over study design, the participants attended two sessions and consumed one treatment at each session. The following treatments were used: (1) blueberry purée and white bread (196.3kcal, 11.4g sugar, 27.7g starch, 5.3g fiber, 6.8g protein, 0.23g fat), and (2) control: sweetened water and white bread (187.3kcal, 11.4g sugar, 27.7g starch, 1.8g fiber, 6.3g protein, 0.23g fat). After an overnight fast, participants arrived to the laboratory and their fasting blood was collected. The participants then consumed a treatment. Subsequent blood samples were collected at 15, 30, 45, 60, 90, and 120min after the treatment. Results: There was an effect of time ( $p=0.002$ ) but no effect of treatment ( $p=0.17$ ) on blood glucose over the 120min. The treatment with blueberries significantly reduced blood glucose by 8.5% at 15 min, and by 6.5% at 30 min compared to the control ( $P<0.05$ ). There was no effect of treatment or time on postprandial total antioxidant capacity in blood plasma as measured using ferryl myoglobin radical-based assay. Conclusion: In healthy adults 19-35 years, HTD processed blueberry beverage consumed with a high carbohydrate meal reduced blood glucose within 30 min after consumption compared to the control with the same level of glycemic carbohydrate. The HTD-processed blueberry beverage did not affect postprandial antioxidant status as measured by trolox-equivalent antioxidant capacity within two hours.



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## **1. Introduction**

The prevalence of diet-related chronic diseases continues to increase amidst various strategies used. Despite the approaches to reduce the prevalence of diet-related chronic diseases such as diabetes mellitus and obesity, related metabolic disorders continue to increase. The World Health Organization (WHO) has indicated healthy diets and physically active lifestyle as sustainable means of preventing diet-related chronic diseases. Therefore, the consumption of food for reasons other than their energy, macro- and micronutrient content, has been on the rise, as more people want to enjoy the medicinal value of food (1).

Existing evidence indicates increased caloric intake, and reduced energy expenditure as some of the causes of the rising prevalence of obesity, diabetes mellitus, and other chronic diseases. A significant contributor to this trend is the increased consumption of sugar-sweetened beverages (2, 3). These beverages contain high amounts of carbohydrates that are easily and rapidly absorbed. They also have low satiety, which encourages further consumption. The excessive intake of glycaemic carbohydrate leads to increased risk of impaired beta-cell function, insulin resistance, and inflammation (3, 4). A healthier option suggested by WHO is increased fruit intake. Fruits and fruit products that provide health benefits without largely increasing sugar intake might be helpful.

Meals that are dense in glycemic carbohydrate and fat might result in a higher oxidative stress (5,6). Postprandial oxidative stress occurs from the pro-oxidative activities of the body after consumption of complex meals that contain inadequate antioxidant sources (6). The beneficial health outcomes of fruits and vegetables include the ability to control oxidative stress and improve blood glucose control. Thus, frequent consumption of fruits is

a means of reducing the risk of developing chronic diseases like heart diseases, stroke, and diabetes mellitus. The antioxidant properties of fruits are evaluated based on their composition. For instance, the high polyphenolic content of blueberries is linked to their antioxidant activities that are beneficial to human health (7).

Dietary fiber, mainly the indigestible carbohydrate portion in plant-based foods, possesses health benefits that have been reported to reduce the rate of glucose absorption (7-9). Polyphenolic compounds such as anthocyanins are organic chemicals with beneficial antioxidant activities that are found in plant-based foods and have also been reported to influence carbohydrate digestion and absorption (10). Antioxidants present in fruits and vegetables can donate electrons to highly reactive species to halt the chain reaction or detoxify free radicals (11). Fruits and vegetables have been suggested by various studies to be good sources of antioxidants (12-14).

Existing evidence has shown the undesirable effect of conventional processing techniques on fiber and polyphenolic content of blueberries and other fruits and vegetables (15). However, other processing techniques including hydrothermodynamic (HTD) processing have been developed to help retain higher proportions of the initial polyphenolic content of the foods. HTD processing involves the grinding, mixing and pasteurization of fruits at the same time in the same tank in a one closed operation unit. HTD technology presents a great advantage as it produces a uniform suspension without discarding skin and seeds, producing a whole fruit purée (16). Due to limited exposure to light, and contact with heat and oxygen, processed products contain high amounts of bioavailable phenolic

compounds. Moreover, phenolics have been found to be highly concentrated in the skins and seeds of fruits that are retained in the purée.

The health benefits of blueberries on blood glucose have been determined to be mainly due to their fiber, anthocyanin and proanthocyanidin contents (7). It has been shown that anthocyanin and proanthocyanidin extracts are able to inhibit  $\alpha$ -glucosidase activity as well as the intestinal glucose uptake (7). The reduced rate of the conversion of starch to glucose leads to reduced postprandial glycaemia.

## **2. Literature Review**

### **2.1 Chronic diseases and their impact on public health and economy**

The increasing prevalence of chronic diseases is of global importance due to the diverse ways they affect various aspects of economic development. Although recent target interventions may have improved the situation, there remains a high proportion of the population that suffer from chronic diseases (17,18). There has been a shift of focus from the prevention of communicable diseases in children to non-communicable diseases in high-income countries like Canada. However, in low-income countries, double burden malnutrition exists. Undernutrition coupled with communicable diseases in children and chronic diseases in adults have increased (18). Overnutrition in adults is a result of increasing pressure of globalization leading to changes in lifestyle, diet and physical activity. A distinguishing reason for the difference in outcome in high-income countries and low-income countries has been attributed to effective public health policies targeting the accessibility of fruits and vegetables in high-income countries (18). High-income countries are gradually succeeding in reducing the level of salt incorporated in processed foods and improving accessibility of fruits and vegetables (19).

The economic burden of chronic diseases involves the cost of direct care and reduced productivity. High proportions of health care budgets are allocated to the management of chronic conditions that could have their onset delayed or even prevented (20). Moreover, chronic diseases reduce the ability of individuals to be actively involved in labor units due to amputation, illnesses, blindness, and mental disorders. According to Gerteis (2004), 86% of total healthcare funds were used in the management of individuals with chronic diseases

(21). About \$176 billion was lost to medical care and \$69 billion to reduced productivity. In 2010, cancer and cardiovascular diseases cost the United States \$157 billion and \$315.4 billion, respectively (22,23). Public Health Agency of Canada (PHAC) reported a similar trend of increasing cost of chronic diseases faster than the economy can keep up with. About 67% of health care costs were spent on chronic diseases. Ding et al. (2016) reported that in 2013, high-income countries spent 80.8% of health care costs and 60.4% indirect cost on physical inactivity and a further loss of \$13.7 billion in productivity losses (24). PHAC stated about \$190 billion as the yearly cost of chronic diseases (25).

WHO reported in 2014 that chronic diseases were the highest cause of death globally and responsible for 68% of approximately 56 million deaths in 2012. About 40% of the deceased were below 70 years. In low and middle-income countries, 82% of deaths were attributed to chronic diseases in addition to the pressing effect of communicable diseases. High-income countries like the United States and Canada however, have made significant progress in reducing conditions contributing to risk factors of chronic diseases (19).

The causes of the onset of chronic diseases have been found to be complex and dependent on interlinked factors of genetics and environment. The World Health Organization reported poor diet, physical inactivity, tobacco, inappropriate use of alcohol, hypertension and raised blood glucose as major chronic disease risk factors (19,26). Tobacco, body mass index, blood pressure, high fasting blood glucose, physical inactivity, diets low in fruits and high consumption of alcohol are the main risk factors for mortality in North America (26). The PHAC also indicated a high prevalence of obesity as a cause of the onset of most chronic diseases in Canada (27).

In determining the economic impact of chronic diseases in the United States of America in 2007, \$1.3 trillion was noted to have been spent on treatment, care, and reduced labor considering that more than half of Americans had one or more chronic diseases (28,29). In projecting the probable economic effect if chronic diseases were not controlled, \$ 4.1 trillion could be lost in 2023. However, the report suggested a 27% reduction in financial burden if chronic diseases were controlled (29).

Cancer, diabetes, cardiovascular diseases and chronic respiratory diseases have been listed as the most common chronic diseases globally (30). Recently, Statistics Canada reported cancer as the leading cause of death (31). On average, within every hour, the Canadian Diabetes Association reported 20 newly diagnosed diabetes cases (32). The continuous increase in the prevalence of chronic diseases has been reported as 4% each year. About 60% of Canadian adults have at least one chronic condition with four out of every five adults being at risk. The trend of chronic diseases being attributed to the elderly is gradually changing in recent times as more conditions were observed in 35-64-year-old adults than in individuals 65 years or more (25). This trend was evident in the prevalence of obesity of 25% in children. A similar trend was reported in individuals over 20 years. Currently, PHAC has put much focus on preventive measures of targeting chronic diseases.

## **2.2 Dietary choices in rising prevalence of Overweight/Obesity as a risk factor of chronic diseases**

Unhealthy dietary choices were reported by WHO as a major modifiable risk factor of chronic diseases (17). Unhealthy dietary choices could lead to elevated blood glucose levels and excess body weight which can gradually increase one's risk of non-communicable diseases (NCDs) (33). Physical inactivity could aggravate this situation. Each year 2.8 million deaths are reported as an outcome of overweight and obesity from possible effects such as increased insulin resistance, and endothelial dysfunction (33).

The Public Health Agency of Canada also indicated a high prevalence of obesity as a cause of the onset of most chronic diseases in Canada (27). Hence more focus has now been directed towards reducing the prevalence of overweight/obesity. Researchers and health workers have introduced various strategies of achieving this aim. However, the aim of reducing prevalence might not have reached desirable levels since overweight/obese continues to increase globally (33).

Currently, there is much focus on preventive measures of targeting chronic diseases (25). Increased intake of sugar-sweetened beverages and low fruit consumption has been associated with increasing prevalence of overweight/obesity, Type 2 diabetes with are both risk factors for developing chronic diseases (34,35).



### **2.3 Consumption pattern of sugar sweetened beverages**

The increased consumption of sugar-sweetened beverages (SSBs) has been noted as a significant contributor to the observed trend of increasing overweight/obesity (34). According to Statistics Canada, the 'other foods' group of Canada's Food Guide contribute 34.7% of sugar consumed daily by Canadians which is mainly derived from consuming SSBs (36). Generally, males consumed more sugar from this category than their female counterparts. Across all age groups, sugar consumption was observed though it reduced with age. The highest sugar consumption level was observed in adolescent boys and lowest in children 1-3 years (36). The associated trend of overweight/obesity has been noted in this population with 61.8% adolescent males self-reporting overweight/obese (37).

Various countries have developed strategies to manage the increase in SSB consumption. Among these include the taxation of sugar-sweetened drinks such as pop, fruit drinks, and sport drinks among others. Other researchers have suggested a change of nomenclature to pathogenic foods instead of the general term junk foods (38). This could deter consumers from consuming those foods and encourage them to make healthier food choices. On February 9, 2016, Dietitians of Canada released a position statement suggesting an excise tax of at least 10-20% on all SSBs to reduce purchase using an economic modelling technique (39). WHO recommends total daily energy contribution from sugar not to exceed 10% (33, 40). However, the average estimate of the total population's energy contribution from sugar was about 21.4% according to the 2004 Canadian community Health survey.

Considering that there is a 15% estimated contribution of energy from SSBs, an effective strategy to reduce their intake might go a long way in reducing total caloric intake

of consumers (40). The Mexican Congress passed a 10% excise tax on high energy foods like sugar-sweetened drinks in September 2013, and this strategy started in January 2014. A year after its implementation, a higher reduction in taxed beverage consumption was observed in low socioeconomic households compared to high socioeconomic households (41).

The Canadian population might have other significant sources of sugar contribution in addition to SSBs. Children 1-8 years had the highest dietary contribution of sugar from milk, fruit, fruit juice and confectionery compared to soft drinks, milk, fruit, fruit juice and confectionery for 9-18 years respectively. Adults obtained most of their sugar from fruit, soft drinks, sugar (brown, white), milk, fruit juice. In adults, sugars and soft drinks contributed 11.4% and 13% to sugar consumption respectively. This eating pattern indicates that significant contribution could be from consumption of sugar-sweetened hot beverages (e.g. tea, coffee) which might be consumed more frequently than soft drinks in adults. SSB consumption reduces with age hence the lowest level of consumption is reported among the elderly. Most age groups consume hot beverages.

To help deter people from consuming SSBs, healthier options should be more accessible. Availability of variety of fruits and fruit products containing high fiber and contributing low glycemic carbohydrate would provide health benefits to the population.

## **2.4 Fruit and vegetable consumption in Canada and around the world**

The WHO recommends 400g of fruit consumption per day or five servings per day. However, the average consumption level of most countries was below the recommended level (42). Centers for Disease Control and Prevention (CDC) reported that between 2007 and 2010, 76% and 87% of American adults did not meet the recommended consumption levels of fruits and vegetables, respectively. As low as 13.1% of adults were reported to have met the recommendations (43). The inability to meet recommendations was attributed to income affecting accessibility and cultural preferences (43).

About 39.5% of Canadians above 12 years were reported to have consumed fruits and vegetables five or more times as recommended by the Canada Food Guide (44). Although this level might appear higher than in the American populace, Statistics Canada indicates a gradual reduction over the years. The trends of the level of consumption reported in 2014 suggested that 46.6% of women met the recommendation as compared to 32.1% for males (44). Younger males were observed to consume more fruits compared to older males (44). A meta-analysis of prospective cohort studies that evaluated the effect of fruit and vegetable consumption on all-cause mortality, cancer and cardiovascular diseases (CVDs) established a positive dose-response relationship. The risk of CVDs was reduced by 6% for every additional serving of fruit per day but was not significantly associated with cancer mortality (35).

## **2. 5 Fruits: Composition and Health Benefits**

Fruits are good sources of dietary fiber, vitamins, minerals, and polyphenolic compounds. Various fruits contain different levels of vitamin C, Vitamin E, beta carotene, and flavonoids. Exogenous antioxidants derived from fruits include polyphenolics and vitamins. For instance, flavones such as isoflavones, flavonones, anthocyanins, catechin and isocathecins have strong antioxidant activity (13). Cao et al. (1998) indicated that onions and molokhia contain high levels of flavones while apples are high in flavonoids (14).

### **2.5.1 Dietary fiber: protective role against chronic diseases**

Dietary fiber includes non-digestible portions of plant-based foods such as pectin, inulin, lignin, resistant starches. They can be either soluble such as pectin, or insoluble such as wheat bran. Soluble fibers are usually fermentable in the colon while insoluble fibers may not be fermentable but possess bulking action. Various studies have reported many health benefits of dietary fiber. However, the consumption rate of dietary fiber remains low. Most people were reported to consume less than half of the recommended intake of 36g/day for men and 28g/day for women (45). The adequate intake level is 14g/1000kcal. Relatively, women were reported to frequently consume high fiber sources such as fruits, vegetables, nuts, whole-grain foods than males (44).

Beyond gastrointestinal function, dietary fiber has a protective role against diabetes, overweight/obesity, and for improved blood glucose control in patients with diabetes and weight loss program in addition to other effects (46-48). In evaluating the effect of intake of

whole grain and fiber on subsequent incidence of type 2 diabetes, Montonen et al. (2003) found an inverse association in a cohort study of 2286 men and 2030 women of 40 – 69 years (47). Lairon et al. (2005) also studied the relation between the type of fiber and risk factors of cardiovascular diseases. It was found that intake of high total dietary fiber and insoluble dietary fiber were associated with reduced risk of overweight and elevated waist-to-hip ratio. Fiber from dried fruits was associated with lower body mass index, and blood glucose concentration (48). Anderson et al. (2004) found that a high fiber diet with moderate carbohydrate intake was associated with lower postprandial plasma glucose in individuals with diabetes mellitus (49).

High fiber foods are more satiating leading to a reduction in within-meal food intake and at the next meal (50). For instance, meals that contain pectin enhanced satiety and delayed gastric emptying (51). Ou et al. (2001) investigated a possible mechanism underlying lower postprandial serum glucose associated with high intake of dietary fiber. Three mechanisms were reported. Firstly, dietary fiber inhibits  $\alpha$ -amylase activity resulting in a reduced rate of conversion of starch to glucose and reduced release of glucose into the blood. Dietary fiber increases viscosity in the small intestine where significant digestion and absorption occurs. Such higher viscosity retards the rate at which glucose diffuses. Finally, dietary fiber molecules adsorb glucose molecules and inhibit diffusion (52).

### **2.5.2 Polyphenolic compounds and their health benefits**

Exogenous antioxidants derived from fruits include bioactive polyphenols. Consumption of dietary polyphenolic compounds could enhance endothelial function and

reduce the risk of chronic diseases (53). However, polyphenols might not only play antioxidant roles but also serve as modulatory signaling molecules (54). Anthocyanins have been identified as responsible for the pigmentation in berries, for example, the blue color in blueberry (55). Usually, anthocyanins are concentrated in the skins of the fruits and enhance the intensity of the color of the fruits (55). Anthocyanins and proanthocyanidins have beneficial biological activities on human health. However, due to their low bioavailability, they are poorly absorbed (8). Health benefits that have been attributed to these polyphenols include enabling the production of detoxification enzymes and antioxidant activity, improved glucose control, improving inflammatory responses, healthy lipid profile, boosting immunity, controlling blood pressure and anti-carcinogenic functions (54-56).

Anthocyanins have positively charged oxygen atoms in their molecules and are therefore able to play significant roles in the anti-oxidative benefits of berries (56). Also, Basu (2011) indicated that anthocyanins counter autoxidation of lipids and protect vascular endothelial cells, and found that blueberry polyphenols protect blood cells from oxidation and free radicals. Various studies have determined numerous roles anthocyanins play. These roles include potential anticancer properties, reducing platelet aggregation therefore enhancing cardiovascular health (55).

Proanthocyanidins were found to slow down the activities of  $\alpha$ -glucosidase thereby reducing the rate at which carbohydrates are hydrolyzed into glucose (7). The inhibition of  $\alpha$ -glucosidase is beneficial in controlling blood glucose because it reduces the incidence of blood glucose spikes that can lead to the development of type 2 diabetes (57). Phenolic acids have been reported by Blacker et al. (2013) to inhibit the glucose uptake in the intestines

after hydrolysis (57). Gallic acid and its esters can scavenge hypochlorous acid and control oxidation of brain phospholipids in ethanol solution. Hydrocynamic acid and its derivatives possess the hydrogen donating ability, and, as such, they are capable of breaking chain reactions and scavenging free radicals (58). Chlorogenic acids also have free radical scavenging roles (59).

The ability of polyphenolics to inhibit oxidative stress from the oxidation of low-density lipoproteins, reduce aggregation of blood platelets and lead to the relaxation of vascular smooth muscles, are the primary outcomes of their anti-oxidative activities (55).

Low fiber intake, high intake of sugar-sweetened beverages, impaired glucose control, and oxidative stress have been identified as the major causes of cardiovascular diseases, diabetes mellitus, and neurodegenerative diseases. These pathological changes occur due to the ability of reactive oxygenated species (ROS) to damage and/or promote cell structure inflammation and insulin resistance (60, 61). Moreover, postprandial oxidative stress increases vascular adhesion and impairs vasodilation. In the development of diabetes, excess caloric intake promotes the formation of ROS (62).

## **2.6 Impaired glucose control**

Blood glucose concentration depends on glucose intake and absorption from the gastrointestinal tract, uptake by peripheral tissues, and production and metabolism in the liver. Insulin, the main blood glucose regulatory hormone stimulates cell glucose uptake to reduce blood glucose concentration. Cell glucose uptake is of use in cell growth and

differentiation activities. Furthermore, insulin stimulates glycogenesis, lipogenesis and protein synthesis while inhibiting glycogenolysis, gluconeogenesis, lipolysis, and protein breakdown. Inhibitory hormones like glucagon, cortisol, growth hormones, thyroxine, catecholamines elevate blood glucose levels via counter insulin mechanisms (63).

Glucose, the digestive end-product of carbohydrates is required for the body's energy needs. Within the first 10 minutes from the start of eating, insulin is secreted by the pancreas in response to the food being eaten. Glucose transporter GLUT4 on the intracellular surfaces are translocated to cell surfaces upon insulin's stimulation (63). Abel et al. (2001) studied the role adipose GLUT4 plays in glucose control since the majority of GLUT4 expression occurs in the muscle. The adipose-tissue-GLUT4-deficient mice used in the study exhibited a 40% reduction in basal glucose uptake compared to the controls. Insulin-stimulated glucose uptake was reduced (highest reduction of 72%) *in vivo*. When skeletal muscles were observed *ex vivo*, neither basal nor insulin-stimulated glucose transport were different from that of the control. However, there was a 40% reduction in insulin-stimulated glucose transport in skeletal muscles *in vivo*. Abel et al. (2001) indicated that skeletal and adipose tissues have complementary roles (64).

Within an hour of eating, blood glucose levels reach their peak value and returns to initial levels in 2-3 hours. This is possible because insulin stimulates cell uptake of glucose (32). However, simple mono- and disaccharides as well as starch are easily digested and have higher ability to increase blood glucose within a shorter period compared to complex structured carbohydrates. Frequent spikes in postprandial blood glucose might overburden



the insulin's secretion and action mechanisms leading to the development of insulin resistance. Muscles and adipose tissues are essential in insulin resistance (60).

## **2.7 Oxidative stress**

### **2.7.1 Formation of reactive species**

The body as a whole system has various maintenance and homeostatic mechanisms. These include energy generation from macromolecules like carbohydrates, fats and proteins. Under physiological conditions, mitochondria serve as the main site of cellular metabolic activities including the break down or build-up of these macromolecules during aerobic cellular respiration (65). These activities can result in the production of reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), due to electron leakage from the electron transport chain in the mitochondria. Reactive species can also result from excess levels of ROS-producing enzymes, drug metabolism, reduced levels of antioxidant and related enzymes and radiations. Reactive species possess one or more unpaired electrons in their orbital, rendering them highly reactive. They comprise nitric oxide (NO), peroxynitrite (ONOO<sup>-</sup>), superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hypochlorite (ClO<sup>-</sup>), and hydroxyl radical (OH<sup>-</sup>), which could be beneficial or detrimental depending on 'the balance' that exists (66).

High carbohydrate meals stimulate oxidation via the tricarboxylic acid cycle. This could easily produce reactive species which can further cause oxidative stress (67).

### 2.7.2 Effect of reactive species

The effect reactive species have on the body depends on the balance between reactive species and both enzymatic and non-enzymatic antioxidants. Reactive species present in low levels could be involved the regulation of cellular function by playing signaling roles and their involvement in immune-mediated defense (68). The imbalance between the generation and elimination of reactive oxygen compounds results in oxidative stress and impaired redox balance (69). The detrimental effect of oxidative stress occurs through its continuous existence in high concentrations in addition to the reduced capacity of the body to eliminate them (68). Therefore, cell exposure to a level of ROS that exceeds its ability to counteract can lead to degradation or modification of the cell's structure and function. Cell components including DNA, proteins, and lipids may then be oxidized by reactive species (70).

ROS can target all classes of biomolecules: lipids, proteins, DNA, and modify their function. Lipids are more susceptible to ROS activity hence a high probability of lipid oxidation (70). The protein structure including the backbone and side chain can both be oxidized. Modification of nucleic acids may result in DNA mutations from DNA-protein crosslinking, strand breaking, and changes in purine and pyridine structure (70,71). Lipid oxidation can degrade the structure of cell membranes and further form reactive aldehydes that bind to the protein and inhibit its normal function (71, 72). This process further leads to the formation of more ROS causing impaired cell structure, impaired DNA structure, and cell death, thereby enhancing the occurrence of malignant cells. Cancer cells have been found to contain higher levels of reactive species than healthy cells (73). Feng (2012) suggested that these changes in the phospholipid component of the cell membrane are a

major cause of neurodegenerative diseases like Alzheimer's disease (74). A similar process has been suggested by Bandeira (2012) to impair pancreatic beta cell function resulting in the pathogenesis of diabetes (75).

In cardiovascular diseases, lipid oxidation begins a series of processes that enhance plaque formation. Complex processes involving angiotensin and the stimulation of angiotensin II receptors in the vascular walls stimulates NADH/NAD(P)H on the walls leading to an imbalance in the redox state, thereby resulting in oxidative stress (76). The occurrence of this process leads to the development of hypertension, a risk factor for cardiovascular diseases. Resulting radicals from the endothelial cells and macrophages oxidize low-density lipoprotein in the subendothelial stream (76). Further calcification at this spot enhances the occurrence of plaques that narrows the circumference of vessels available for blood flow. Moreover, the plaque could rupture and block blood vessels in the heart or brain.

However, exceeding reactive species reduction mechanisms could result in reductive stress. This condition could decrease the cell's sensitivity to apoptotic signals, leading to necrosis and further to tumor formation (69).

### **2.7.3 Reactive species in obesity and related diseases**

During the tricarboxylic acid (TCA) cycle, electron donors such as reduced nicotinamide adenine dinucleotide (NADH) and 1,5-dihydroflavin adenine dinucleotide (FADH<sub>2</sub>) are produced (67). In the presence of high levels of glucose metabolism within the cell, the rate of glucose oxidation via the TCA increases, contributing to the generation of electron donors in the mitochondrial electron transport chain. This process enhances the

donation of electrons to molecular oxygen and the production of superoxide. Similarly, resulting acetyl-CoA from the oxidation of free fatty acids (FFAs) and beta-oxidation of fatty acids enter the TCA, yielding further FADH<sub>2</sub> and NADH (67). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has been reported to be involved in the production of nutrient based reactive species (77).

The plasma membrane contains NADPH oxidase that converts molecular oxygen to superoxide. Adipocytes with high FFAs generate more ROS. However, NADPH oxidase inhibitor controls the process. Therefore, accumulated fat in obesity enhances NADPH oxidase activity thereby producing high levels of ROS. The outcome from obese mice has indicated increased mRNA expression of NADPH oxidase in adipocytes, not in liver or muscles (77).

As a defense mechanism, adipocytes contain high levels of antioxidant enzymes to control ROS activity. Similarly, in obese subjects, the expression and activity of antioxidant enzymes such as catalase, superoxide dismutase (SOD1), and glutathione peroxidase (GPX) were observed to be decreased in the adipose tissue (78). This aggravates oxidative stress in obese individuals, increasing their risk of metabolic syndrome. Adipocytokines, like tumor necrosis factor alpha (TNF $\alpha$ ) and adiponectin, are biologically active substances produced in adipocytes that are involved in obesity-related diseases (79). The decreased levels of adipocytokines in obese individuals contribute to insulin resistance and atherosclerosis. For instance, adiponectin has insulin-sensitizing and anti-atherogenic effects and its levels are inversely correlated with systemic oxidative stress, therefore altered levels increase the risk of developing diabetes mellitus and cardiovascular diseases (73, 77).

Insulin response in ROS-exposed adipocytes has been seen to be reduced in glucose uptake, translocation of glucose transporter 4 (GLUT4), and lipogenesis (80). Stress signals including p38 mitogen-activated protein kinase were activated by excessive oxidative stress in affected adipocytes. Moreover, hydrogen peroxide was detected to act as a second messenger in insulin signals in adipocytes (81). Therefore, lasting existence of ROS affects glucose metabolism and insulin action (73, 80).

In limiting the effect of reactive species to its defensive and signaling role, antioxidant molecules work in synergy to minimize free radical reactivity with cellular components. Endogenous antioxidant compounds (e.g., urate, glutathione, ubiquinone, and thioredoxin) along with some proteins (ferritin, transferrin, lactoferrin, caeruloplasmin) bind to transition metals to avoid the initiation of oxidative reactions (73). Exogenous sources such as dietary antioxidants include vitamin C, vitamin E, and phytochemicals. Zinc, manganese, and selenium also play significant roles in the activity of antioxidant enzymes (73).

## **2.8 Antioxidants**

Antioxidants are compounds that are capable of preventing the oxidation of an oxidizable substrate when present in a lower concentration than the substrate (66). Antioxidant activities can help control or prevent the detrimental effect of oxidative stress, thereby reducing the onset of DNA mutations, malignant transformations, and cell damages and consequently reducing the occurrence of cancer and other non-communicable diseases (70).

The mechanism of action of antioxidants include the prevention of reactive species, capturing radicals already formed, removal of damaged biomolecules and the reduction of modification in cell metabolism. Some antioxidants are depleted during their protective actions involving removing pro-oxidative metal ions, scavenging chain initiating radicals, and breaking chain reactions (82).

Two classes of antioxidants include endogenous and exogenous species. Endogenous antioxidants like glutathione are produced within the body and enhance redox homeostasis. Exogenous antioxidants, on the other hand, are consumed through diet and supplements. Ascorbic acid, vitamin E, carotenoids and phenolic compounds are examples of exogenous antioxidants (68, 83). The two classes of antioxidants complement each other in their protective roles. Excess intake of antioxidants through supplements can lead to the formation of pro-oxidants (83).

### **2.8.1 Assessing antioxidant status**

Several *in vitro* methods are used to assess the antioxidant capacity of biological specimens. The most commonly used methods include the ferric reducing ability of plasma (FRAP), the Trolox Equivalent Antioxidant Capacity (TEAC), the oxygen radical absorbance capacity (ORAC) assay, and the total radical trapping parameter (TRAP) (84).

#### **Trolox equivalent antioxidant capacity (TEAC) assay**

This method measures the ability of a sample to reduce the 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) radical. The absorbance of the resulting solution is measured with a spectrophotometer. The ABTS radical results from the reaction between

ABTS and ferrylmyoglobin radical species after activation of metmyoglobin with hydrogen peroxide ( $H_2O_2$ ). A modified assay uses a preformed ABTS radical from the oxidizing ABTS with potassium persulfate (85).

The coefficients of variation (CV) recorded by Rice-Evans and Miller (1994) were 0.54–1.59% and 3.6–6.1% for intra- and inter-assays. Although the TEAC is based on the ability of the sample to reduce ABTS radical, ferrylmyoglobin radicals might also be reduced. Therefore, the antioxidant capacity might be underestimated since only the reduction of ABTS radical is determined. Wang et al. (2004) suggested that there is a lack of correlation between TEAC and other methods such as ORAC and FRAP. That may be due to such underestimation. (85).

### **Ferric Reducing Ability of plasma**

Formation of ferrous ions from the reaction between plasma samples, TPTZ (2,4,6-tripyridyl-s-triazine) and  $FeCl_2 \cdot 6H_2O$ , is used as a measure of the antioxidant activity. Under low pH conditions, an intense blue color results from the reduction of ferric-trotripyridyltriazine ( $Fe^{III}$ -TPTZ) complex to the ferrous ( $Fe^{II}$ ) form. The increase in absorbance by the resulting solution is measured at 593 nm in low pH conditions. The FRAP reagents contains 10 mM of TPTZ solution in 40 mM HCl, 1 mL 20 mM  $FeCl_2 \cdot 6H_2O$ -  $H_2O$ , and 10 mL of 0.3 M acetate buffer with pH 3.6 (86).

This method is reproducible, rapid and easy to perform. Moreover, a linear relationship exists between the reaction and molar concentration of antioxidants. However, it has limited application since antioxidants containing thiol (SH) groups cannot be measured using FRAP. Benzie and Strain (1996) reported an intra-assay coefficient of variation (CV) less than 1.0% and 3.0% between runs at 100 – 1000  $\mu\text{mol/liter}$ .

### **Total radical trapping parameter (TRAP) assay**

In this assay, peroxy radicals resulting from the interaction between 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and peroxidizable materials in the sample are determined. On addition of AAPH, oxygen required for the oxidation of oxidizable materials is measured. This process is inhibited by antioxidant compounds in the sample analyzed during the induction period. By comparing it to an internal standard, the induction period provides an estimate of the antioxidant capacity (87). The inability of the oxygen electrode to maintain stability over the required period poses a challenge for the utilization of this method.

Several methods have been developed based on this principle. Such methods include the luminol-based assay which measures emitted light from the production of peroxy radicals from the oxidation of luminol by AAPH (88). Aejmelaeus et al. (1997) reported CV for intra- and inter-assay as 2% with n = 10 (89). Similarly, the dichlorofluorescein-diacetate (DCFH-DA) based assay measures fluorescence of dichlorofluorescein (DCF) from oxidizing DCFH-DA with peroxy radicals. CV values of 3.4% and 4.6% were reported for intra- and inter-assay (90). Resulting experimental values are significantly affected by some pathological conditions like acute myocardial infarction.

### **Oxygen radical absorbance capacity (ORAC) assay**

In this method, phycoerythrin is used as the oxidizable protein substrate and either AAPH is used to produce peroxy radical or  $\text{Cu}^{2+}$ - $\text{H}_2\text{O}_2$  for hydroxyl radical. It comprises exhaustion of the free radical (91). The area under curve (AUC) method is used to quantify



the inhibition percentage and the period used by antioxidants to inhibit free radical action. Low reproducibility of the lag phase has been indicated to be challenging considering the non-zero order kinetics of quenching the resulting phycoerythrin fluorescence. However, the AUC technique gives an advantage over utilizing only the lag phase. ORAC assay indicates the ability of antioxidants to inhibit free radical activity directly hence high specificity. This assay takes about 70 min to obtain results (91).

Phycoerythrin based assays use AAPH to produce peroxy radicals or  $\text{Cu}^{2+}$ -ascorbate for hydroxyl radicals with R-phycoerythrin (R-PE) as the oxidizable substrate. In the presence of peroxy radicals, the fluorescence produced by the oxidizable substrate is reduced in a linear relationship with time. The antioxidant capacity is determined by comparing the length of lag phase to a Trolox standard. However, the lag phase has been reported as challenging with plasma or serum samples (92).

Ghiselli et al. (1995) reported that after 80% of R-PE fluorescence was quenched, reduction ceased to be linear with time. However, a CV of 0.96% was reported, indicating high reproducibility (93).

Presently, there is no standardized method for determining the antioxidant capacity in plasma. However, FRAP, ORAC, and TEAC are usually used.

## **2.9 Blueberries: varieties, composition, and processing**

Blueberries belong to the family *Ericaceae*, subfamily *Vacciniaceae*, genus *Vaccinium* and subgenus *Cyanococcus*. They are perennial flowering plants with indigo-colored berries. Blueberries, in general, are common in the Eastern part of North America and can also be

found in Europe, Argentina, and Australia (94). Blueberries are native to North America – specifically, the wild lowbush blueberries are highly produced in Canada, with Atlantic Canada as the major producer contributing about 85% of total production. Blueberries have been identified to be a healthy food choice along with other fruits and vegetables due to their chemical composition (95,96).

### **2.9.1 Blueberry varieties**

Several varieties of blueberries include wild growing lowbush blueberry (*Vaccinium angustifolium*), cultivated northern highbush blueberry (*Vaccinium corymbosum*), and rabbiteye blueberry (*Vaccinium ashei*). Bilberries (*Vaccinium myrtillus*) are predominant in Europe and they possess characteristics similar to the northern highbush blueberries, albeit having a different pulp color. The Southern highbush blueberry is a cultivar made from interbreeding northern highbush and rabbiteye blueberries (94, 96). Mostly in North America, as a seasonal plant, southern highbush blueberry was cultivated around May to September and picked in August and September of the harvesting year (96).

### **2.9.2 Composition of blueberries**

The raw fruits contain about 85.5% moisture, 12.3% carbohydrate including 2.6% dietary fiber and 3.1% glucose, 3.3% Fructose, 1.2% protein, 0.8% fat, and other components like vitamins, minerals, and polyphenols (8,95).

Flavonoids and non-flavonoids are the two major polyphenolics present in blueberries. These components confer physical and chemical properties to the berries. Flavonoids include anthocyanins, proanthocyanidins, flavonols and catechins while non-

flavonoids include chlorogenic acid, hydroxycinnamic acids and phenolic acids like gallic acid (97,98). Anthocyanins are more concentrated in the peels with proanthocyanidins distributed in both the peels and seeds. Hydroxycinnamic acids are usually found in the flesh.

Blueberries contain total dietary fiber of 2.6g per 125ml which is the serving size (99). Some research correlated increased dietary fiber intake with improved health outcomes. In a prospective study, dietary fiber was associated with reduced risk of mortality from cardiovascular diseases (100). In both individuals with and without diabetes mellitus, it was observed to help improve blood glucose control thereby preventing or managing insulin resistance (101, 102). These outcomes are beneficial in significantly reducing the risk of cardiovascular diseases, gastrointestinal diseases, diabetes and improving immune function.

The composition of blueberries is influenced by the season of cultivation, variety, climate, degree of ripeness, light exposure, among other variables (98,103). Kalt (2001) determined seasonal variation of polyphenolics; 30% in lowbush and 35-40% in highbush. Connor et al. (2002) also reported that different levels of anthocyanins were observed in the same cultivars grown at different locations and between different cultivars grown at the same location, suggesting a combined effect of genotype and environment on anthocyanin levels

### **2.9.3 Bioactive compounds in blueberries**

The main bioactive compounds in blueberries are flavonols like quercetin and myricetin, ascorbic acid, hydroxybenzoic acids such as gallic acids and protocatechuic acids, hydroxycinnamic acids like caffeic acids, ferulic acids and coumaric acid, anthocyanins, resveratrol, and pterostilbene (104,105). These components exist in varying concentrations

depending on genetics, agricultural practices, storage conditions, processing techniques, and method of analysis used.

Capra (2006) and Prior et al. (1998) reported an average of 10 mg per 100 g of ascorbic acid in blueberries. Lowbush blueberries might contain levels as high as 16.4 mg/100 g while the highbush might contain 5 – 10 mg/100 g fresh fruits and 3 mg/100 g ascorbic acid (106,107). Oxygen level and temperature significantly impact ascorbic acid content of fruits such that there was 27% reduction in fresh fruits stored at 20°C for eight days (108).

Similarly, Ehlenfeldt and Prior (2001) reported wide variation in total phenolic content of cultivars from 48 to 304 mg/100 g (109). Santos, Almeida, Lopes, & DeSouza, (2006) pointed out that phenolic acids, such as chlorogenic acid exist in high amounts in blueberries, and hence enhance their anti-inflammatory effects (110).

The range of total flavonoids in blueberries was reported to be 2.5 – 387.48 mg/100 g of fresh fruits, which put blueberries among foods with highest antioxidant activity (111). Myricetin and quercetin were reported by Taruscio et al., (2004) as the major blueberry flavonols (112). The European blueberry (bilberry) has been found to contain the highest concentration of these flavonols.

The biological protective roles of blueberries have been suggested to be due to the chemical structure of these condensed tannins since blueberries contain mainly proanthocyanidins. These roles include ameliorating the antioxidant environment in pancreatic  $\beta$ -cells, and reducing intestinal glucose absorption by inhibiting intestinal  $\alpha$ -glucosidase activity (113). In addition, a protective role on the neuronal function of various

brain regions has been attributed to several tannins. Anthocyanins also contribute to anti-inflammatory and antioxidant benefits of blueberries. Lowbush species have over 1000 mg/100 g anthocyanin content while highbush have up to 800 mg/100 g (114,115).

The bioactive components in blueberries have been known to reduce postprandial glycaemia and insulin in Type 2 diabetes patients fed bilberry extract equivalent to 50g fresh bilberries. Participants consumed the bilberry extract which contained 36% w/w anthocyanins and 75g polycal liquid or an anthocyanin free placebo with 75g glucose and 75g polycal liquid (116). However, contrasting results were reported by Qin et al., (2009) and Zhu et al. (2013), suggesting that the level of bioactivity is influenced by anthocyanin bioavailability (117,118).

Upon supplementation with blueberry extracts or anthocyanins, the metabolites were detected in the blood, liver, kidney and ocular tissues; anthocyanins were found in the serum after blueberry consumption and a significant increase in serum antioxidant status was observed *ex vivo*. This indicates that blueberry extracts, anthocyanins, and their metabolites have the ability to produce metabolic effects in the body (119,120).

The antioxidant properties *in vitro* might not be mainly due to vitamins, fibers or antioxidant minerals; rather anthocyanins have been indicated to be responsible for antioxidant properties. Ascorbic acid was found to enhance the antioxidant capacity of blueberries by 10% by Barberis et al. (2015) while Borges et al. (2010) indicated that anthocyanins contributed about 84% (121,122).

## **2.10 Effect of fruit processing on their composition**

The methods and principles of processing influence the health enhancing capacity of fruits and vegetables. The chemical composition, bioavailability, and activity of bioactive compounds are determined by the cultivation practices, postharvest storage, handling and processing methods used (123).

Processing techniques such as blanching, juice pressing, and clarification, however, impact the characteristics of fruits. Processing is typically needed to enhance shelf-life, safety, quality, to produce a high variety of products, and for economic benefits. For instance, antioxidant properties of fruits and vegetables could be lost, improved, or retained after processing (15,124). There is also the possibility of the formation of new compounds that possess antioxidant or pro-oxidant properties, like in Maillard reaction products (123) or loss of vitamin C while cooking (15). On the other hand, the oxidation state of some bioactive compounds changes during processing to enhance their ability to donate hydrogen atoms (125). Blanching inhibits the activities of oxidative enzymes in fruits and vegetables, preserves color, and prevents loss of naturally occurring antioxidants (125).

### **2.10.1 Effect of conventional processing on blueberries**

Considering that blueberries like other fruits are perishable and seasonal, storage and processing is necessary to extend their shelf-life. Storage can either increase or reduce anthocyanin content based on storage conditions. Phenolics are water soluble hence anthocyanins in soft broken skins could leach out. High wax skin blueberries might be able

to retain higher amounts during storage (126). Common storage techniques, freezing and freeze-drying have been found to have minimal effect on anthocyanins (127).

Conventional processing techniques usually used for berries compromise their beneficial components. For instance, anthocyanin function is affected by exposure to temperature and oxidation is likely to happen during conventional processing (127). During conventional processing, fruits may be peeled and thermally treated leading to the destruction of some polyphenols. Pressing, filtration, clarification, freeze drying and juice concentration could reduce fiber content as well as anthocyanin levels which are concentrated on the skins and seeds (128).

Contradicting evidence exists for the effect of temperature on blueberry polyphenolics. Brownmiller (2008) determined an 8% reduction in non-clarified juice and 5% in clarified juice (128). However, Skrede (2000) found 4% increase in anthocyanin concentration after heat treatment at 90°C for 90 sec. Evidence from Skrede (2000) suggested that the application of heat enhanced the activation of anthocyanins during processing (129).

During conventional processing, berries could be macerated, enhancing polyphenol oxidase (PPO) activity. Anthocyanins might not be the primary substrate of PPO, however *o*-quinones from oxidized phenolics could stimulate degradation of anthocyanins. In some cases, PPO can degrade the structure of anthocyanins (130). Moreover, pectinases added to macerate to enhance juice yield could promote phenolic degradation if pectinases contain  $\beta$ -glucosidases. The thermosensitive nature of anthocyanins might cause reduction of

anthocyanin content when exposed to heat above 70°C, but are not affected by heat up to 60°C (127).

Alternate processing techniques including hydrothermodynamic (HTD) processing have been developed to help retain much of the initial polyphenolic content of the foods (16). HTD processing involves the grinding, mixing and pasteurization of fruits at the same time in the same tank in a closed operation unit. HTD processed blueberry products possess enhanced shelf-life and higher polyphenol content compared to conventionally processed products. Moreover, HTD produces a whole purée with a high level of fiber and polyphenol retention (16).

Healthy dietary choices significantly reduce the risk of developing obesity, diabetes mellitus among other chronic diseases. These healthy dietary choices include increasing fruit consumption to benefit from their fiber and bioactive components. Fruits such as blueberries undergo various conventional processing techniques that potentially reduce their beneficial components. Emerging methods including HTD processing provide an alternative that helps retain bioactive compounds and fiber. However, it is unknown whether HTD processed blueberry products have beneficial effects on blood glucose control and antioxidant status in humans.



### **3. Rationale and Objectives**

#### **3.1 Rationale**

The rate of chronic diseases is increasing and its effect on quality of life and level of productivity affects various aspects of economic development. Low fruit and vegetable consumption has been found to be a risk factor for the onset of chronic diseases. Polyphenolic compounds and dietary fiber in blueberries have been found to have beneficial health outcomes. These components affect both starch digestion and glucose uptake in the blood, thereby controlling the level of blood glucose in the serum after consumption. Antioxidant activity of polyphenols in berries also has a significant role in controlling oxidative stress.

However, conventional fruit processing methods result in a significant reduction in the levels of fiber and polyphenols in blueberries. Hence, optimum health benefits from their consumption may not be realized. HTD processing offers a better alternative with respect to the retention of polyphenols. However, whether this leads to improved glycemic control and reduced antioxidant status *in vivo* is not known. Therefore, this study aimed to evaluate the metabolic response of the consumption of HTD processed berry products.

### **3.2 Objectives**

To evaluate the effect of HTD processed blueberry purée consumed with a starch-based food on postprandial blood glucose and antioxidant status in human adults.

### **3.3 Hypothesis**

The consumption of HTD-processed blueberry product served with a high glycemic food will result in a delayed blood glucose response and increased serum antioxidant status compared to the control with the same level of available carbohydrate.

## **4. Methods**

### **4.1 Study design**

A randomized controlled crossover study design was used. Each participant received the treatment, which comprised HTD blueberry purée and white bread, and the control made of sweetened water and white bread in a randomized order. The treatment was assigned “1” and the control assigned “2” in no particular order. Microsoft Excel was used to generate the random order.

### **4.2 Inclusion criteria**

Eligible participants were healthy adults aged 19-35, non-smokers, with a normal body mass index (BMI), and with no known allergies to blueberries and white bread. Participants were not taking any medications known to influence glucose metabolism and antioxidant status. Individuals with no developmental or learning problems that would prevent them from participating in the study as designed were included.

### **4.3 Recruitment and Screening**

Advertisement cards and fliers were handed out to individuals at Mount Saint Vincent University, and an advertisement was posted on Kijiji to invite interested individuals to participate (Appendix 1). An initial telephone screening questionnaire (Appendix 2) screening was carried out to determine the eligibility of interested individuals before the in-person screening in the laboratory. The in-person screening involved anthropometric measurements (height, body weight) and body composition measurement using a Tanita body composition analyzer TBF 300A. Eligible participants were required to complete a baseline questionnaire (Appendix 3) to obtain further information mainly on a health status.

Female participants filled out a menstrual cycle questionnaire (Appendix 4). Female participants had experimental sessions during the follicular phase of the menstrual cycle to avoid possible interference of glucose metabolism that occurs in the luteal phase (131).

#### **4.4 Ethical consideration**

The study and all procedures were reviewed and approved by the University Research Ethics Board.

The procedure, benefits and potential risks associated with the study were explained to the participants after which their informed consent was obtained on the consent form (Appendix 4). Verbal descriptions were also given to ensure participants clearly understood the study components and requirements.

#### **4.5 Experimental session**

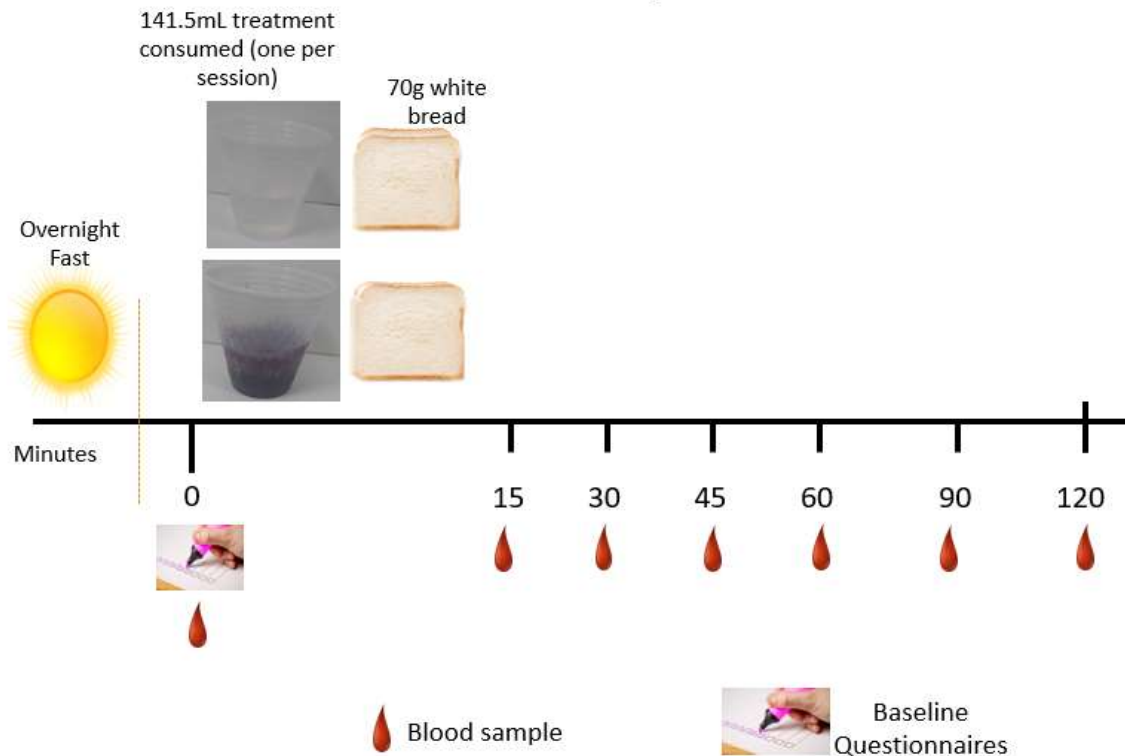
The procedure for the experimental sessions was similar to the method used by Törrönen et al. (2010) (7,132). Recruited participants were required to fast for 12 hours before the morning of the test day. Sessions were held in the mornings, starting from 8 am to 9:30 am. Participants remained in the laboratory during the 2-hour postprandial period. Blood samples were taken by registered nurses using aseptic techniques and in a hygienic environment.

On arrival to the laboratory on the morning of the session, participants filled out the Health and Activity Questionnaire (Appendix 6) to determine if they met the overnight fast and provided information of what the previous day's activities were and if they might affect their ability to do the sessions. Afterward, fasting blood glucose level was measured. If fasting blood glucose was higher than 6.0mmol/L, the session was rescheduled for another day, and if it was 6.0mmol/L or less, participants proceeded to the nurse station.

Baseline blood samples were then taken by registered nurses using venipuncture and vascular access devices into heparin tubes and serum separating tubes (SST). The participants then proceeded to the feeding cubicles to consume either the treatment or control depending on the order from randomization. Afterward, the participants returned to the test area for the following 2 hours while blood samples (8.5 ml each) were collected at 15, 30, 45, 60, 90 and 120 min after start of consuming treatment, similar to the method used by Törrönen et al. 2010 (7).

The samples in the heparin and serum separating tubes were processed according to directions by the manufacturer. Samples were then stored at -80°C in working aliquots of 150µL and 250µL for heparin tubes and SST, respectively.

**Figure 4.5:** Study protocol



#### 4.6 Dietary treatments

Fully ripened wild blueberries provided by PEI Berries Ltd, Montague, PEI, Canada were processed by Dr. Alex Martynenko's research group at the Faculty of Agriculture, Dalhousie University in Truro, NS, using the HTD processor. Blueberries were heated to 95°C and held for 0-2 min retaining mean anthocyanin content of  $87.47 \pm 4.79$  mg/100 g (Table 2).

White bread samples were freshly baked in the lab using a Sanyo SBM-201 Automatic breadmaker. White bread was made from 540g white wheat all-purpose flour, 360g water, 4.8g dry yeast (Fleischmann Traditional active dry yeast), and 4.8g salt. The weighed ingredients were added to bread pan in the order above as directed by the manufacturer. The Basic (normal) option of the SANYO “Bread Factory Plus” Bread Maker, SBM-201 was selected. Freshly baked samples were frozen at -20°C and thawed overnight for use in upcoming sessions.

#### **4.7 Analytical Methods**

The nutrient composition of bread (Table 5.1.1) was conducted by Maxxam Analytics (Mississauga, ON). Analysis of the anthocyanin content, brix, polymeric color, and viscosity were conducted at Dr. Alex Martynenko’s lab at the Department of Agriculture, Dalhousie University in Truro.

Blood glucose and total antioxidant capacity over the 120-minute sampling period for both treatments were analyzed. Blood glucose was analyzed electrochemically with YSI 2300 STAT Plus Glucose & Lactate Analyzer. This analyzer measures D-glucose at the enzyme sensor to produce hydrogen peroxide. With the aid of glucose oxidase, glucose is oxidized to produce hydrogen peroxide and D-Glucono- $\delta$ -Lactone. These are proportional to the concentration of the glucose in the sample.

The total antioxidant activity was determined using the CS0790 Sigma-Aldrich Antioxidant Assay Kit (Sigma-Aldrich, Missouri, United States). The TEAC assay is based on the ability of a sample to reduce the 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) radical. The absorbance of the resulting solution is measured with a

spectrophotometer at 405nm. The ABTS radical results from the reaction between ABTS and ferrylmyoglobin radical species after activation of metmyoglobin with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

#### **4.8 Statistical Methods**

Student t-test was used to assess the difference between the treatments for postprandial blood glucose and total antioxidant capacity. The student paired t-test was conducted with Microsoft Excel 2013.

Statistical analysis was conducted with SAS University Edition 9.2 (Statistical Analysis Systems, SAS Institute, Cary, NC, USA). Total, incremental and net incremental area under the curve (AUC) values were determined using the participants' fasting blood sample values as the baseline level. Repeated measure two-way ANOVA tests were conducted to compare treatments, time, sex, total antioxidant activity and blood glucose values with significance set at p-value  $\leq 0.05$ . When the effect of treatment was significant, Tukey-Kramer's test was used to perform a post hoc analysis.



## 5. Results

### 5.1 Composition of Dietary treatments

The nutrient composition of white bread and blueberry purée is shown in Table 5.1.1 and Table 5.1.2 respectively. The dietary treatments included either 150 g HTD blueberry purée or 141.5 ml water with 10 g of sucrose, similar sugar as in the purée, and two slices of bread (70g) (Table 5.1.3). Some physical and chemical properties of the HTD processed purée are shown in Table 5.1.2

Table 5.1.1: Nutrient composition of white bread

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Nutrient composition	Per 70g (2 slices)
Energy (kcal)	154
Total Carbohydrate(g)	31.5
Sugar (g)	1.4
Starch(g)	27.7
Fibre(g)	1.8
Glycaemic carbohydrate (g)	30.4
Protein (g)	6.3
Fat (g)	0.23

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Table 5.1.2: Nutrient composition of Blueberry purée

Nutrient composition	Per 150g
Energy (kcal)	72.6
Total Carbohydrate(g)	19.1
Sugar (g)	10
Starch(g)	0
Dietary fiber(g)	3.5
Glycaemic carbohydrate (g)	15.5
Protein (g)	0.5
Fat (g)	0

Table 5.1.3: Composition of dietary treatment

Nutrient composition	Treatment (Blueberry purée and white bread)	Control (Sweetened water and white bread)
Weight (g)	220	151.5
Energy (kcal)	196.3	187.3
Total Carbohydrate(g)	44.4	40.9
Sugar (g)	11.4	11.4
Starch(g)	27.7	27.7
Dietary fiber(g)	5.3	1.8
Glycaemic carbohydrate (g)	39.1	39.1
Protein (g)	6.8	6.3
Fat (g)	0.23	0.23

Table 5.1.4: Physical and chemical characteristics of HTD blueberry beverage

Characteristics	Mean $\pm$ SD
Anthocyanin content (mg/100g)	87.47 $\pm$ 4.79
Polymeric color (%)	16.70 $\pm$ 0.32
Brix (%)	11.67 $\pm$ 0.12
Viscosity (Pa. s)	1.43 $\pm$ 0.02*

\*viscosity was measured at fixed shear rate of 20 s<sup>-1</sup> at temperature 20<sup>o</sup>C.

## 5.2: Baseline characteristics of participants

With a total of 25 participants, the average age of participants involved in the study was  $23.3 \pm 4.4$  years. 12 of the 25 participants were females with a mean age of  $22.9 \pm 4.1$  years and  $23.6 \pm 4.8$  years for the 13 male participants. The mean body mass index (BMI) for all participants was  $22.3 \pm 2.2$  kg/m<sup>2</sup>, similar for males recording  $22.4 \pm 1.8$  kg/m<sup>2</sup> and  $22.1 \pm 2.6$  kg/m<sup>2</sup> for female participants. The basal metabolic rate (BMR) recorded was  $1608.2 \pm 231.8$  kcal -  $1801.6 \pm 144.3$  kcal for males and  $1608.2 \pm 231.8$  kcal for females.

Female participants recorded a higher body fat of  $22.3 \pm 9.5\%$ , and  $13.4 \pm 4.1\%$  for males resulting in an average of  $17.9 \pm 8.5\%$  for all participants. Similarly, the fat mass assessed in males was  $9.7 \pm 3.2$  kg and  $13.8 \pm 7.5$  kg for females with a total mean of  $11.7 \pm 6.0$  kg. Consequently, the fat-free mass was  $62.2 \pm 7.1$  kg and  $44.6 \pm 4.6$  kg for males and females, respectively, with a total of  $53.4 \pm 10.7$  kg. Total Body Water was observed to be higher in males ( $45.5 \pm 5.2$  kg) than in females ( $32.7 \pm 3.4$  kg), thereby resulting in a mean of  $39.1 \pm 7.9$  kg for all participants.

Three interested participants could not complete sessions due to difficulties encountered by the nurses while conducting venipuncture for blood collection. Another could not continue due to change in commitments. These individuals were compensated for any sessions attended and later replaced with other participants. The data of one female participant was not used due to missing data points.

**Table 5.2:** Baseline characteristics of participants

Variable	MALES (n = 13)	FEMALES (n = 12)	TOTAL (n = 25)
Age (years)	23.6 ± 4.8	22.9 ± 4.1	23.3 ± 4.4
Weight (kg)	72.4 ± 7.8	58.6 ± 9.2	65.8 ± 10.9
Height (cm)	179.6 ± 6.6	162.6 ± 5.9	171.5 ± 10.6
BMI (kg/m <sup>2</sup> )	22.4 ± 1.8	22.1 ± 2.6	22.3 ± 2.2
Body Fat (%)	13.4 ± 4.1	22.3 ± 9.5	17.9 ± 8.5
BMR (KCAL)	1801.6 ± 144.3	1414.7 ± 98.3	1608.2 ± 231.8
Fat Mass (Kg)	9.7 ± 3.2	13.8 ± 7.5	11.7 ± 6.0
Fat Free Mass(kg)	62.2 ± 7.1	44.6 ± 4.6	53.4 ± 10.7
TBW	45.5 ± 5.2	32.7 ± 3.4	39.1 ± 7.9

*Data are Mean ± SD, n = 25. Abbreviations: BMI, body mass index; BMR, basal metabolic rate;*

*TBW, total body water*

### 5.3 Postprandial Blood Glucose

There was a main effect of time ( $P = 0.002$ ) but no significant effect of treatment ( $P = 0.2$ ) and sex ( $P = 0.2$ ) on mean cumulative blood glucose concentrations in this sample over 120 minutes (Table 5.1). No Treatment\*Time ( $P = 0.1$ ), Time\*Sex ( $P = 0.08$ ), Treatment\*Sex ( $P = 0.5$ ) and Treatment\*Time\*Sex ( $P = 0.7$ ) effects on mean cumulative blood glucose concentration were observed. The mean blood glucose change from the baseline level was significantly higher when participants consumed the control treatment. As time proceeded, there was an initial increase from baseline to peak, which then reduced gradually to 120 min. The cumulative mean blood glucose concentration of the 25 participants involved in this study were not significantly different within two hours of consuming both treatments. The mean cumulative BG concentration recorded after 2 hours of consumption of the blueberry purée was  $5.4 \pm 0.6$  mmol/L and  $5.4 \pm 0.7$  mmol/L for the control.

Overall, blood glucose concentration for the water treatment peaked to 6.6 mmol/L at 30 min and decreased gradually to 4.5 mmol/L at 120 min. For the blueberry treatment, the blood glucose peaked to 6.4 mmol/L at 45 min and diminished steadily to 4.5 mmol/L by 120 min.

The baseline blood glucose for both the treatment (blueberry beverage and white bread) and the control (sweetened water and white bread) were  $4.6 \pm 0.4$  mmol/L and  $4.7 \pm 0.4$  mmol/L, respectively, which were not significantly different ( $P = 0.49$ ). However, at 15 minutes, the blood glucose was significantly higher after the control ( $5.6 \pm 0.8$  mmol/L) compared to the treatment with blueberry ( $5.2 \pm 0.5$  mmol/L) ( $P = 0.005$ ). A similar pattern was observed at 30 min: blood glucose after control was higher ( $6.6 \pm 1.0$  mmol/L) compared to blueberry

treatment ( $6.2 \pm 0.7$  mmol/L) ( $P=0.05$ ). There was no significant difference between the treatments over the 45-120 min period.

In assessing the area under the glucose response curve, there was a significant difference ( $P = 0.008$ ) at 30min. The area observed 30min after consuming the treatment was  $85.25 \pm 7.82$  mmol/L\*min/L mmol and  $92.1 \pm 11.6$  mmol/L\*min/L for the control. No significant differences were observed at 60min and 120min after consumption of the treatment and control (Table 5.3).



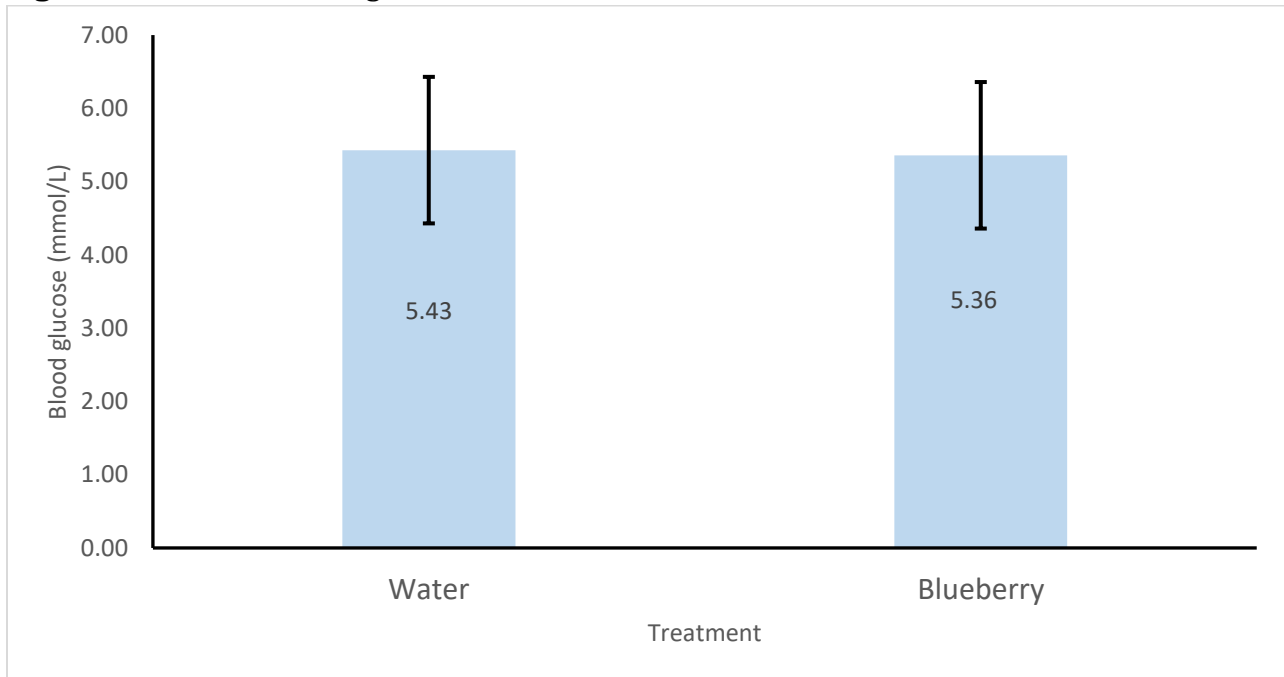
Table 5.3: Postprandial Blood Glucose Response

	Treatments		Effect of treatment (P-value)
	Blueberry beverage and white bread	Sweetened water and white bread	
Mean Blood Glucose over 30min (mmol/L)	5.3 ± 0.4	5.6 ± 0.6	0.01
Mean Blood Glucose over 60min (mmol/L)	5.6 ± 0.6	5.8 ± 0.7	0.2
Mean Blood Glucose over 120min (mmol/L)	5.4 ± 0.6	5.4 ± 0.7	0.6
Mean Blood Glucose Change from Baseline over 30min (mmol/L)	1.1 ± 0.6	1.5 ± 0.7	0.006
Mean Blood Glucose Change from Baseline over 60min (mmol/L)	1.3 ± 0.7	1.4 ± 0.8	0.3
Mean Blood Glucose Change from Baseline over 120min (mmol/L)	0.9 ± 0.6	0.9 ± 0.7	0.9
Glucose Total AUC over 30min (mmol/L*min/L)	85.3 ± 7.8	92.1 ± 11.6	0.008
Glucose Total AUC over 60min (mmol/L*min/L)	90.7 ± 16.8	89.7 ± 19.6	0.8
Glucose Total AUC over 120min (mmol/L*min/L)	646.1 ± 80.7	646.9 ± 90.4	1.0
Glucose Net Incremental AUC over 30min (mmol/L*min/L)	16.4 ± 8.3	22.5 ± 10.7	0.006
Glucose Net Incremental AUC over 60min (mmol/L*min/L)	25.7 ± 21.8	20.1 ± 18.9	0.6

Glucose Net Incremental AUC over 120min (mmol/L*min/L)	94.9 ± 71.7	89.5 ± 86.9	0.7
Glucose incremental AUC (mmol/L*min/L) over 30min	16.5 ± 8.1	22.5 ± 10.7	0.007
Glucose incremental AUC (mmol/L*min/L) over 60min	22.3 ± 15.2	20.9 ± 17.9	0.6
Glucose incremental AUC (mmol/L*min/L) over 120min	104.1 ± 63.3	109.6 ± 69.8	0.7

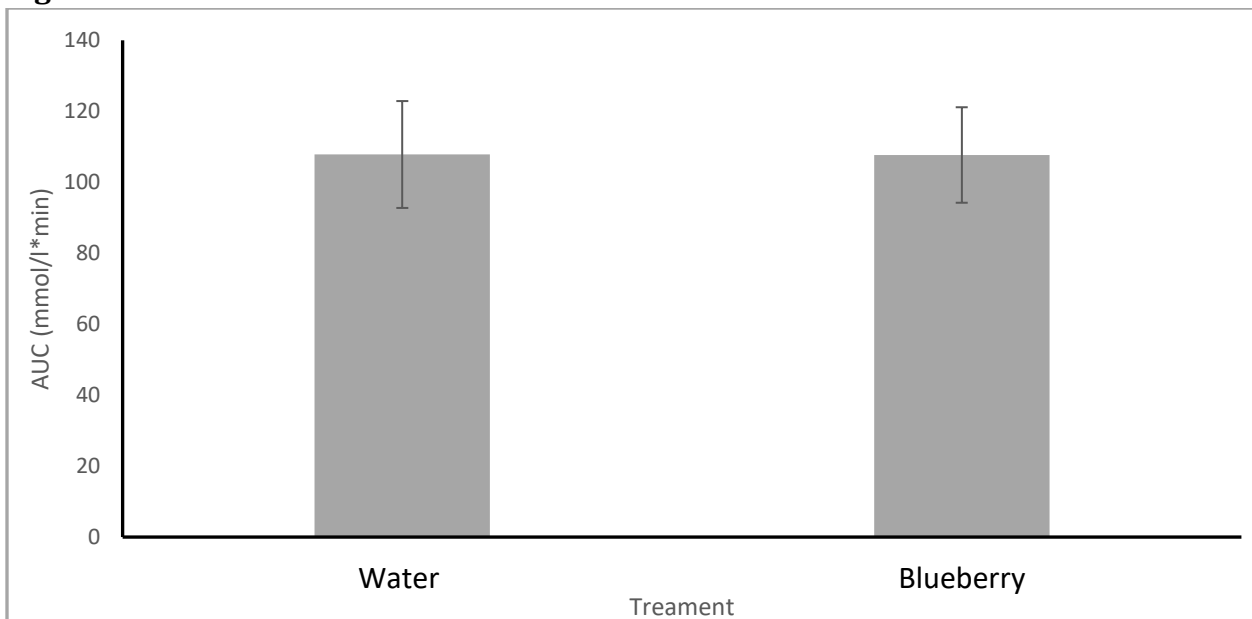
*Data are Mean ± SD, n = 25. Abbreviations: AUC, Area under the curve. P-values are from Student paired t-test*

**Figure 5.3.1:** Mean Blood glucose over two hours



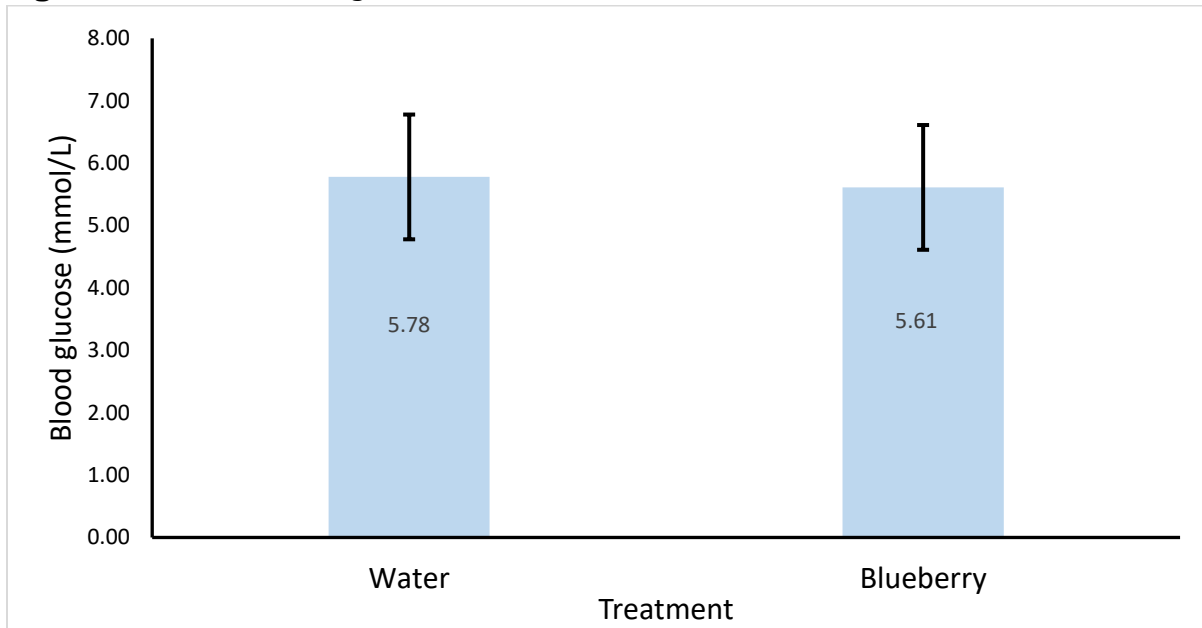
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.2$ ), Effect of time ( $p = 0.002$ ) Effect of sex ( $p = 0.2$ ), Treatment\*time ( $p = 0.1$ ). P-values are from repeated measures ANOVA

**Figure 5.3.2:** Mean BG total AUC over two hours



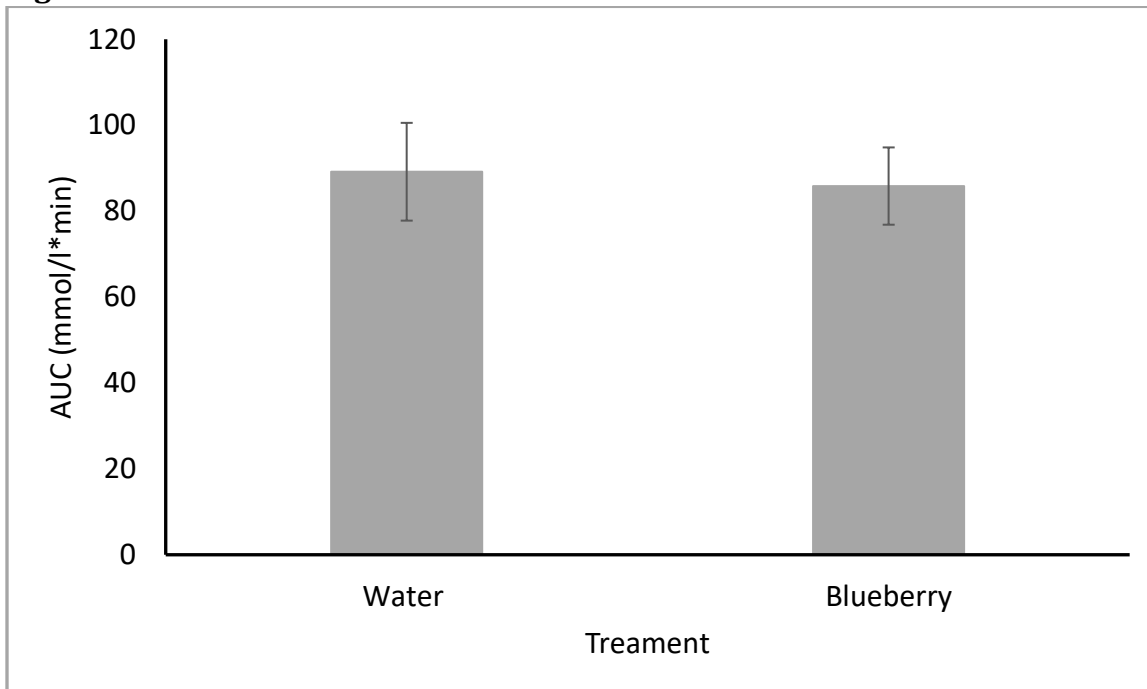
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.1$ ), Effect of time ( $p < 0.0001$ ) Effect of sex ( $p = 0.7$ ), Treatment\*time ( $p = 0.07$ ) P-values are from repeated measures ANOVA

**Figure 5.3.3:** Mean blood glucose over 60min



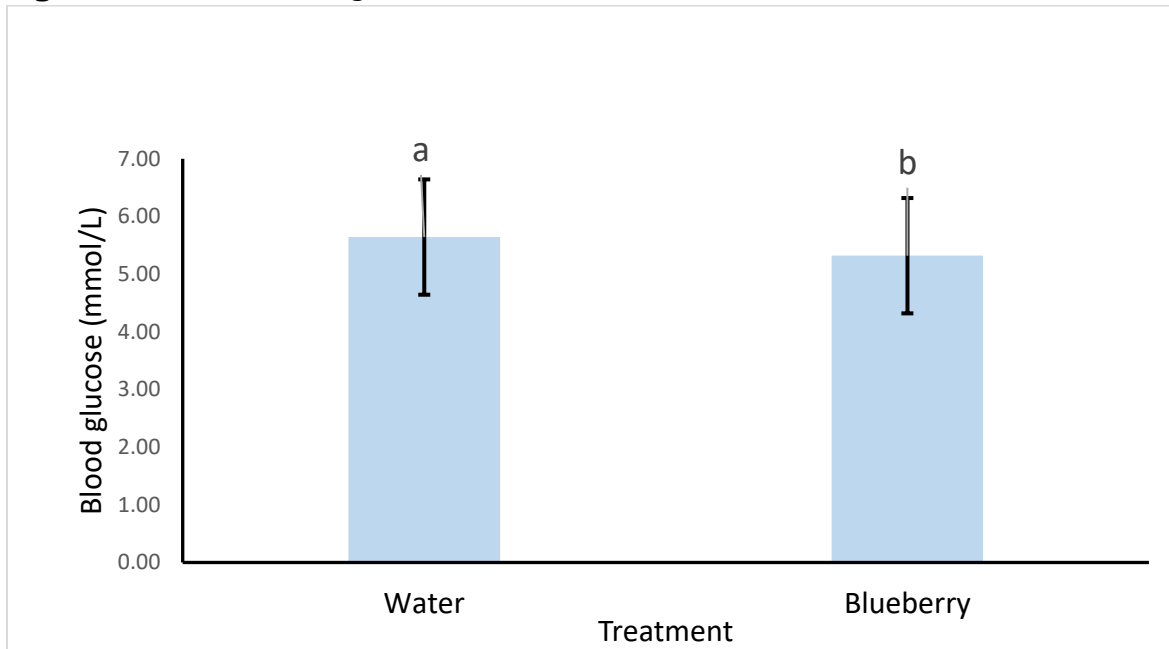
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.2$ ), Effect of time ( $p < 0.0001$ ) Effect of sex ( $p = 0.9$ ), Treatment\*time ( $p = 0.3$ ) P-values are from repeated measures ANOVA

**Figure 5.3.4:** Mean BG total AUC over 60min



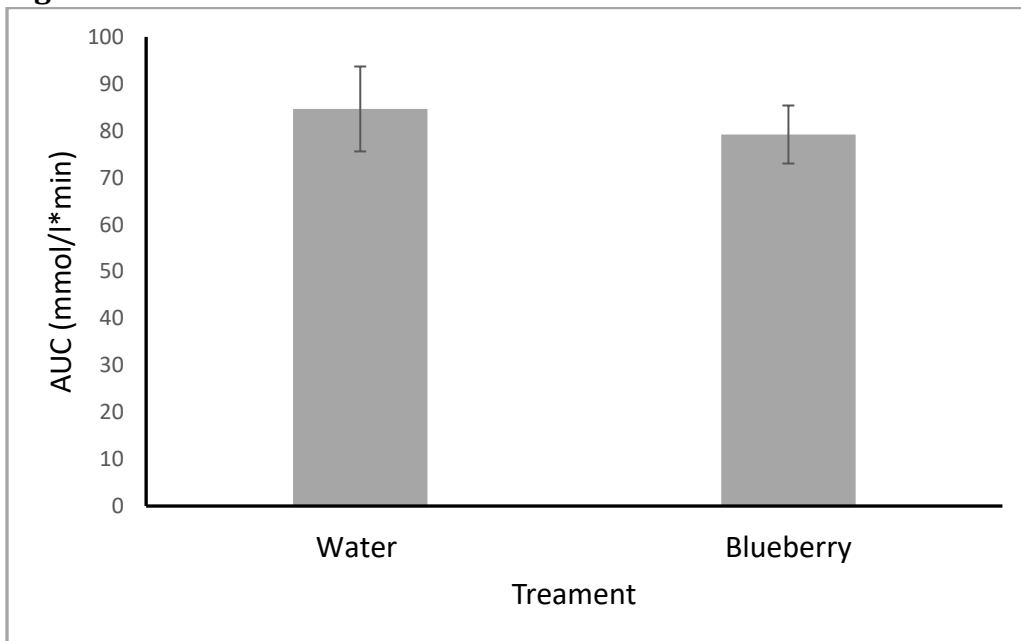
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.06$ ), Effect of time ( $p < 0.0001$ ) Effect of sex ( $p = 0.3$ ), Treatment\*time ( $p = 0.2$ ) P-values are from repeated measures ANOVA

**Figure 5.3.5:** Mean blood glucose over 30min



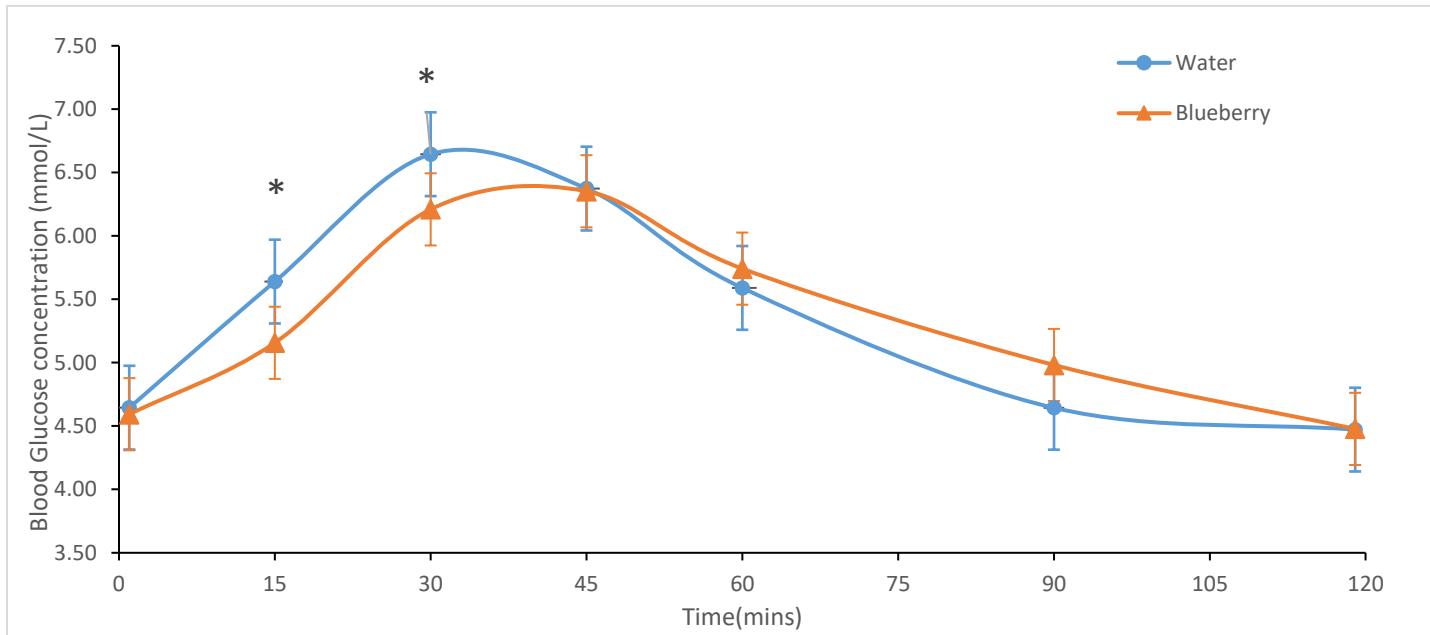
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.4$ ), Effect of time ( $p < 0.0001$ ) Effect of sex ( $p = 0.4$ ), Treatment\*time ( $p = 0.07$ ) Different alphabet indicate significance ( $P \leq 0.05$ ) P-values are from repeated measures ANOVA

**Figure 5.3.6:** Mean BG total AUC over 30min



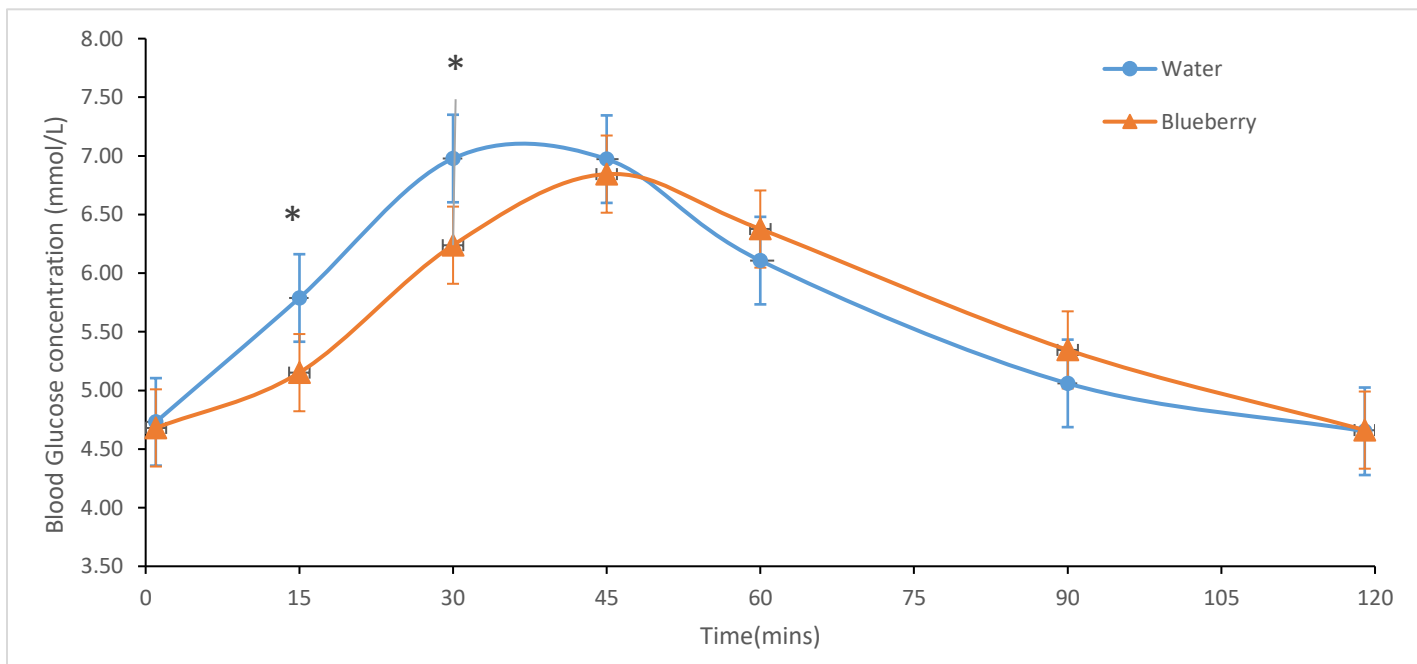
Data are mean  $\pm$  SD, n=25 Effect of treatment ( $p = 0.7$ ), Effect of time ( $p < 0.0001$ ) Effect of sex ( $p = 0.8$ ), Treatment\*time ( $p = 0.1$ ) P-values are from repeated measures ANOVA

**Figure 5.3.7:** Mean blood glucose over two hours



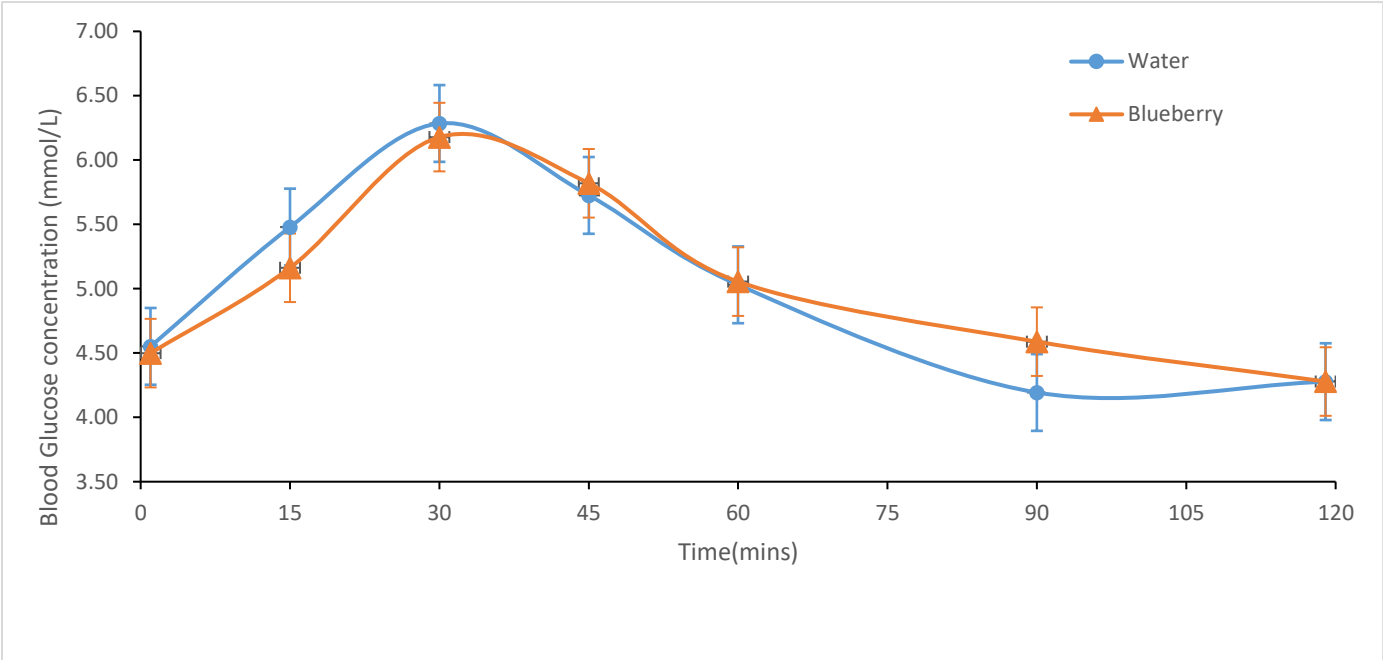
Data are mean  $\pm$  SD, n=25. \*Indicates statistically significant difference between the treatments ( $P \leq 0.05$ ).

**Figure 5.3.8:** Mean Blood glucose over two hours for male participants



Data are mean  $\pm$  SD, n=13. \* Indicates statistically significant difference between the treatments ( $P \leq 0.05$ ).

**Figure 5.3.9:** Mean Blood glucose over two hours for female participants



Data are mean  $\pm$  SD, n= 12.

#### 5.4: Total Antioxidant Capacity (mM Trolox)

The total antioxidant capacity observed when the blueberry beverage was consumed was not significantly different from when the sweetened water (control) was consumed. Similarly, the change of TAC from the baseline value was not significantly different between treatments. Overall, no significant main effect of treatment ( $P = 0.80$ ) or time ( $P = 0.32$ ) was observed. As time proceeded, there was no particular TAC trend for either treatment or control and within sex groups. However, there was an effect of sex of the participants ( $P = 0.01$ ); different patterns were observed among males and females. At 15 min, females recorded a significantly higher TAC when blueberry purée was consumed compared to the control. On the other hand, males recorded significantly lower TAC at 45 min when BB was consumed. There was no significant Treatment\*Time ( $P = 0.7$ ), Treatment\*Sex ( $P = 0.03$ ), and Treatment\*Time\*Sex ( $P = 0.7$ ) interaction effects within this sample over the 120min.

There were no significant differences between the total AUC, incremental AUC and net incremental AUC after consumption of treatments at 60 min and 120 min.



Table 5.4: Total Antioxidant Capacity

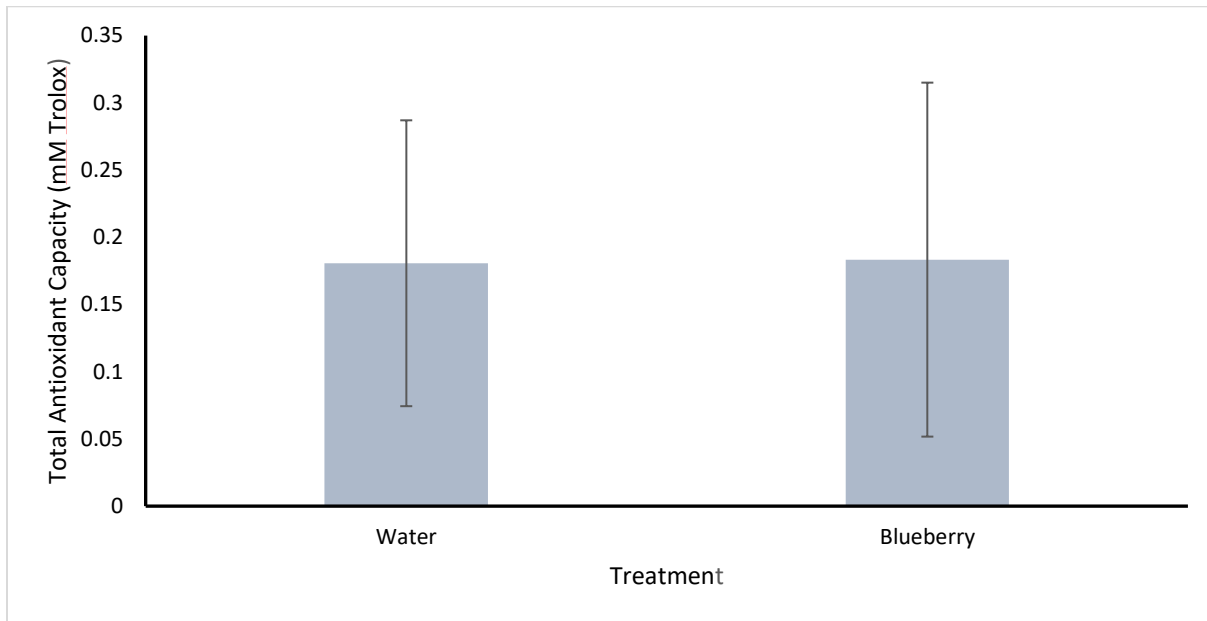
	Treatments		Effect of treatment (P-value)
	Blueberry beverage and white bread (Mean $\pm$ SD)	Sweetened water and white bread (Mean $\pm$ SD)	
Mean Blood TAC over 30min (mM Trolox)	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.8
Mean Blood TAC over 60min (mM Trolox)	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.8
Mean Blood TAC over 120min (mM Trolox)	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.9
Mean Blood TAC Change from Baseline over 30min (mM Trolox)	0.001 $\pm$ 0.07	0.0006 $\pm$ 0.08	1.0
Mean Blood TAC Change from Baseline over 60min (mM Trolox)	0.004 $\pm$ 0.07	0.002 $\pm$ 0.08	0.9
Mean Blood TAC Change from Baseline over 120min (mM Trolox)	-0.004 $\pm$ 0.06	-0.004 $\pm$ 0.08	1.00
TAC Total AUC over 30min (mM Trolox*min)	2.7 $\pm$ 2.0	2.7 $\pm$ 1.8	1.0
TAC Total AUC over 60min (mM Trolox*min)	2.6 $\pm$ 1.8	3.0 $\pm$ 2.5	0.4
TAC Total AUC over 120min (mM Trolox*min)	22.2 $\pm$ 14.7	21.8 $\pm$ 13.07	0.8
TAC Net Incremental AUC over 30min (mM Trolox*min)	0.1 $\pm$ 1.0	0.1 $\pm$ 1.1	1.0
TAC Net Incremental AUC over 60min (mM Trolox*min)	0.2 $\pm$ 1.2	0.6 $\pm$ 2.8	0.4

TAC Net Incremental AUC over 120min (mM Trolox*min)	1.0 ± 6.8	1.3 ± 8.9	0.9
TAC incremental AUC over 30min (mM Trolox*min)	0.4 ± 0.6	0.4 ± 0.7	0.9
TAC incremental AUC ) over 60min (mM Trolox*min)	0.5 ± 0.76	1.1 ± 2.4	0.2
TAC incremental AUC over 120min (mM Trolox*min)	3.5 ± 4.7	4.4 ± 6.1	0.4

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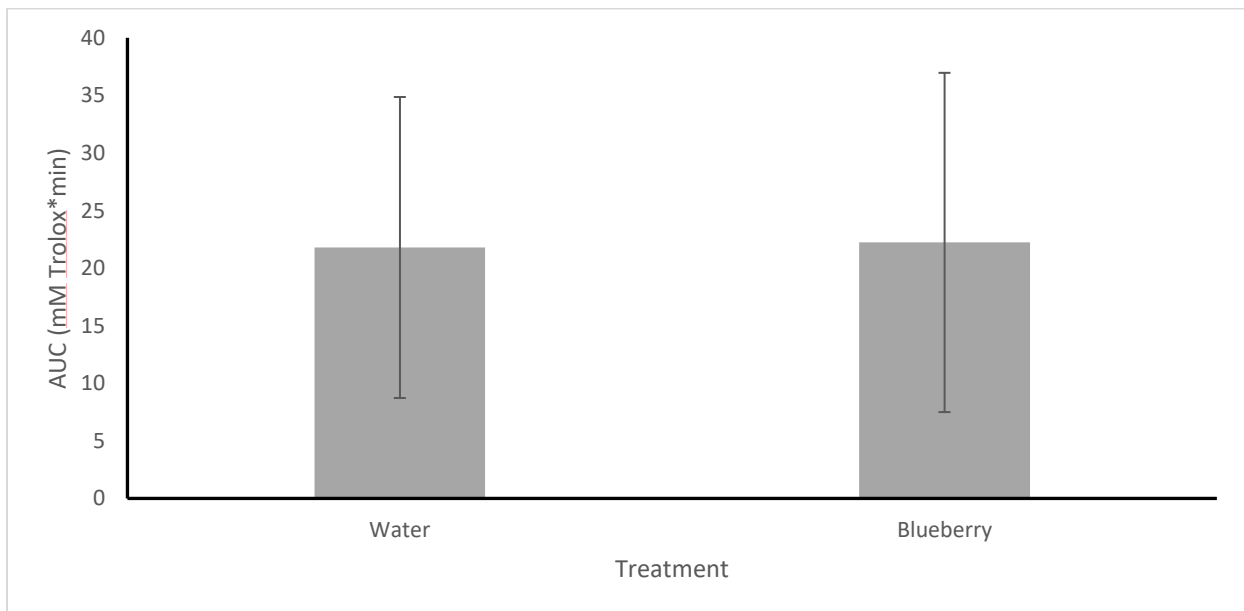
*Data are Mean ± SD, n = 25. Abbreviations: TAC, total antioxidant capacity; AUC, Area under the curve. P-values from Student t-test was conducted with Excel 2013*

**Figure 5.4.1:** Mean TAC over two hours



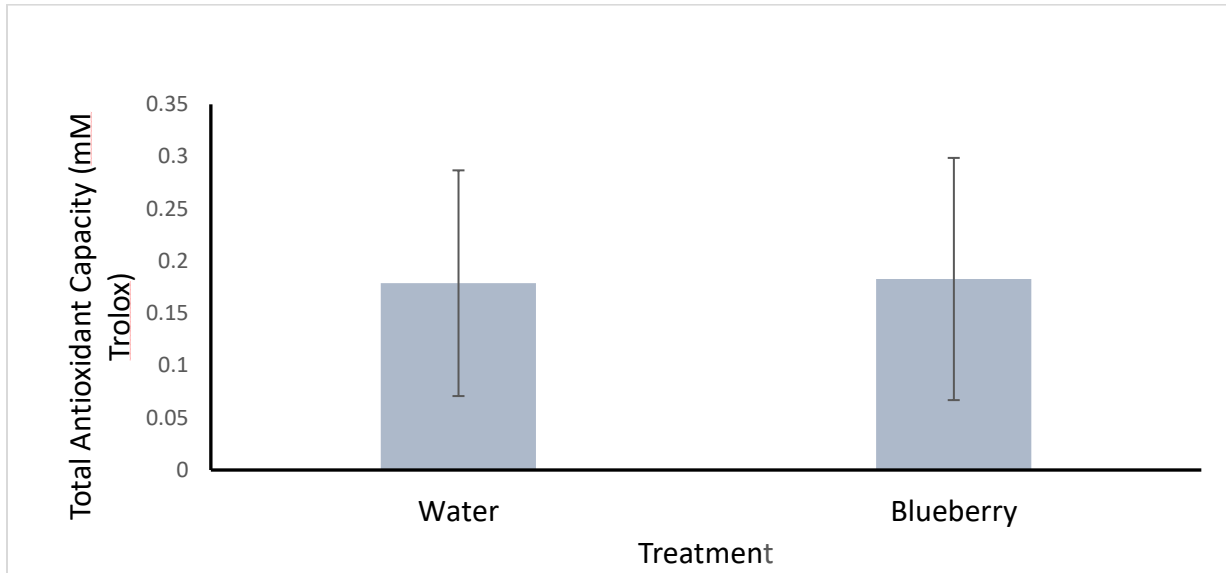
Data are mean  $\pm$  SD, n=25 Effect of treatment ((P = 0.80), Effect of time (P= 0.32) Effect of sex (p = 0.19), Treatment\*time (p = 0.7) P-values are from repeated measures ANOVA in SAS

**Figure 5.4.2:** Mean TAC AUC over two hours



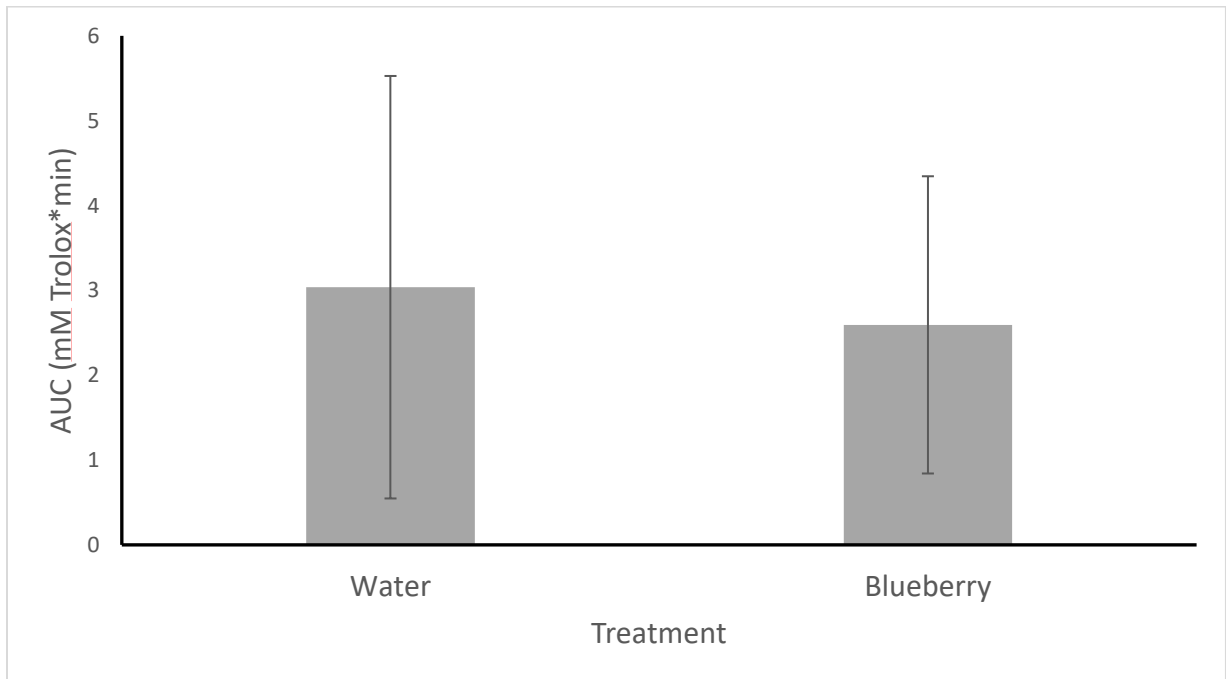
Data are mean  $\pm$  SD, n=25 Effect of treatment ((P = 0.14) Effect of time (P= <0.0001) Effect of sex (p = 0.7), Treatment\*time (p = 0.07) P-values are from repeated measures ANOVA in SAS

**Figure 5.4.3:** Mean TAC over 60min



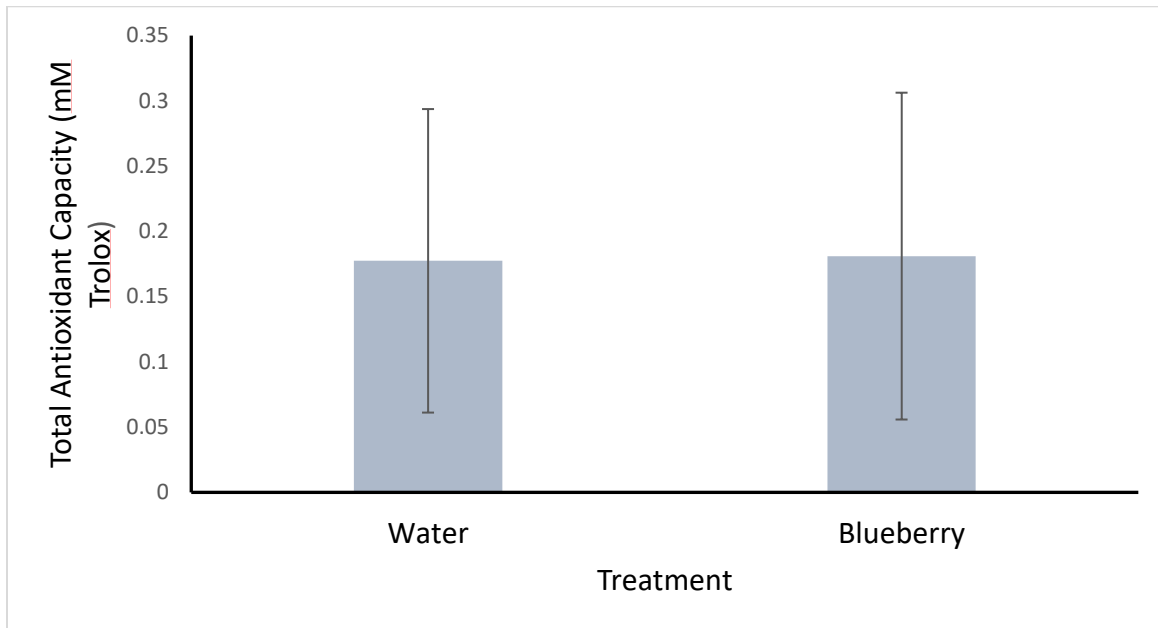
Data are mean  $\pm$  SD, n=25 Effect of treatment (( $P = 0.83$ ), Effect of time ( $P = 0.17$ ) Effect of sex ( $p = 0.005$ ), Treatment\*time ( $p = 0.87$ ) P-values are from repeated measures ANOVA in SAS

**Figure 5.4.4:** Mean TAC AUC over 60min



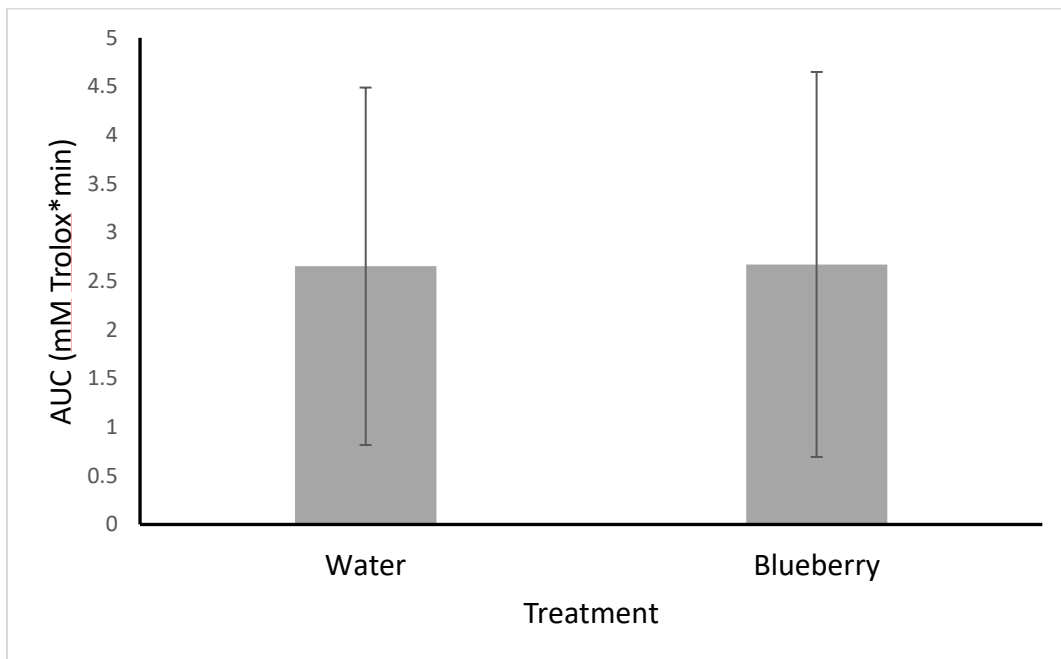
Data are mean  $\pm$  SD, n=25 Effect of treatment (( $P = 0.72$ ), Effect of time ( $P < 0.0001$ ) Effect of sex ( $p = 0.7$ ), Treatment\*time ( $p = 0.1$ )

**Figure 5.4.5:** Mean TAC over 30min



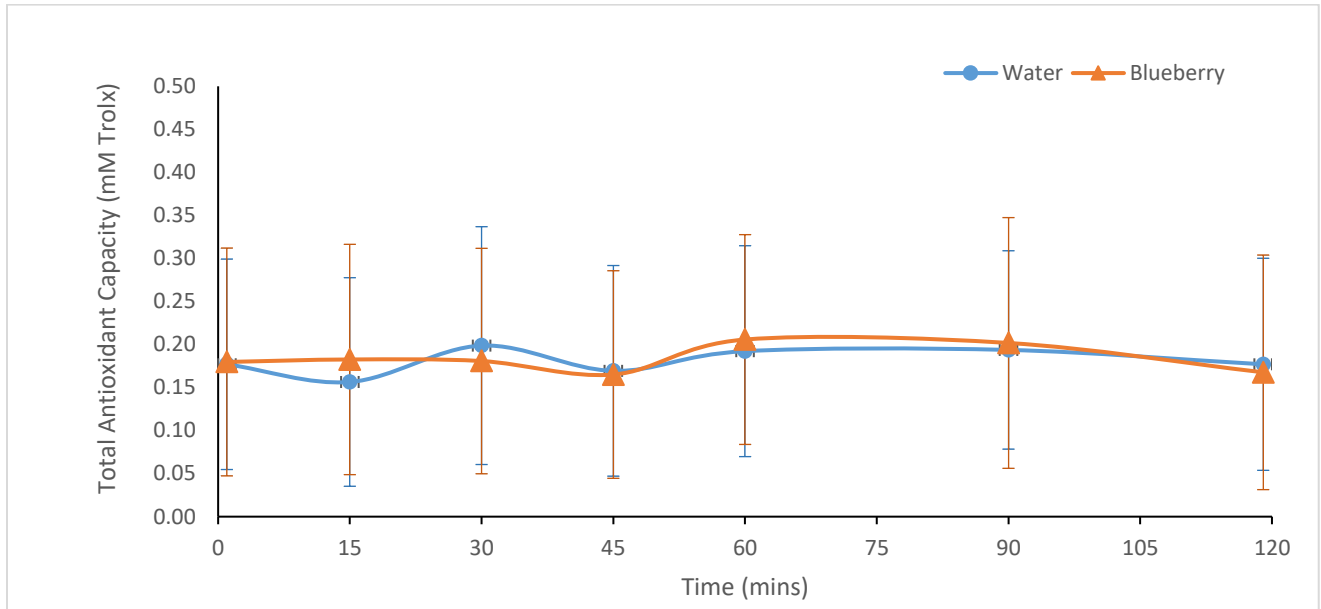
Data are mean  $\pm$  SD, n=25 Effect of treatment ((P = 0.66), Effect of time (P= 0.34) Effect of sex (p = 0.01), Treatment\*time (p = 0.38) P-values are from repeated measures ANOVA in SAS

**Figure 5.4.6:** Mean TAC AUC over 30min



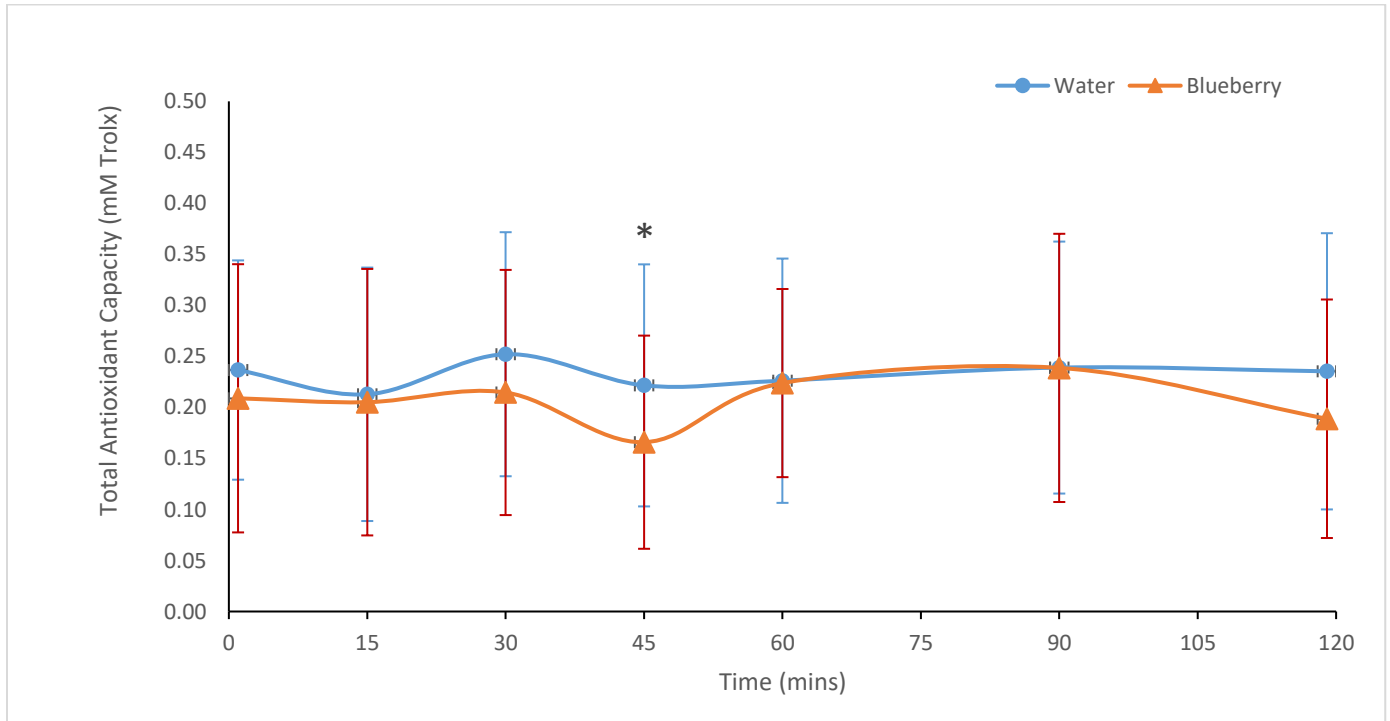
Data are mean  $\pm$  SD, n=25 Effect of treatment ((P = 0.72) Effect of time (P<0.001) Effect of sex (p = 0.77), Treatment\*time (p = 0.1) P-values are from repeated measures ANOVA in SAS

**Figure 5.4.7:** Overall mean total antioxidant capacity over 120mins



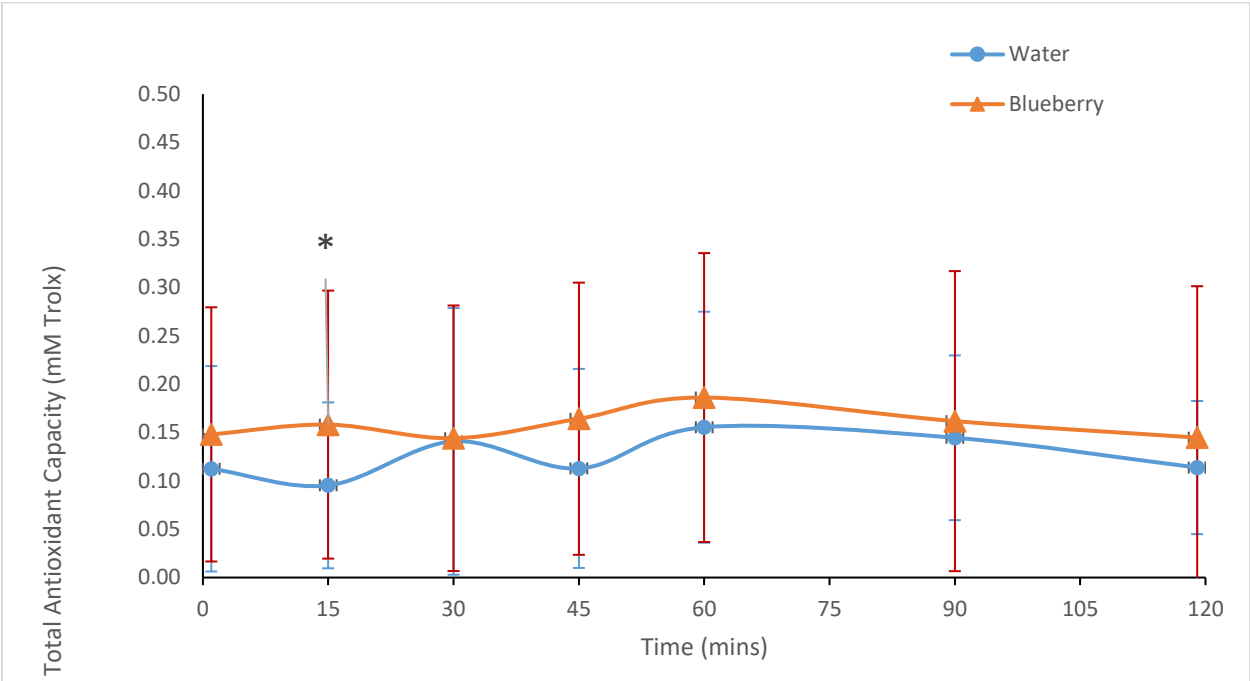
Data are Mean  $\pm$  SD, n = 25

**Figure 5.4.8:** Mean total antioxidant capacity over 120mins for male participants



Data are Mean  $\pm$  SD, n = 12\* indicate significance  $p \leq 0.05$

**Figure 5.4.9:** Mean total antioxidant capacity over 120mins for female participants



Data are Mean  $\pm$  SD, n = 12 \* indicate significance  $p \leq 0.05$

## 6. Discussion

### 6.1 Postprandial Blood Glucose

In determining the effect HTD processed blueberry beverage (BB) has on postprandial blood glucose (BG), mean blood glucose concentration over 2 hours was not different when both treatments were consumed. There was a significant effect of time ( $P = 0.002$ ) but no significant effect of treatment ( $p = 0.17$ ) on the blood glucose concentrations. No significant treatment\*time interaction was observed. Kay and Holub (2002) reported a similar trend of a significant effect of time and no significant treatment\*time interaction (10) after participants consumed either a blueberry supplement or a control supplement with a high fat meal. Both the blueberry supplement (100g freeze-dried powder) and control supplement (matched with digestible carbohydrate profile of blueberry supplement) were consumed with a high-fat meal (one Egg McMuffin<sup>®</sup>, one Sausage McMuffin<sup>®</sup>, and two hash brown patties). However, Kay and Holub (2002) observed a significant effect of treatment after monitoring serum glucose concentration (SGC) for 4 hours after consumption of treatment compared to the 2-hour period that was used in the current study. Serum glucose concentration was observed to be higher in the blueberry-treatment group at 3 ( $p = 0.03$ ) and 4 hours ( $p = 0.03$ ) (10) although the same amount of digestible carbohydrates were provided in both groups. The difference in outcome of both studies could be attributed to the amount of blueberries provided. Participants in the current study consumed 150g of purée whereas 100g freeze dried blueberries (500-650g whole blueberries) were provided by Kay and Holub (2002). On the other hand, Clegg et al. (2011) studied the effect of berries on postprandial glycaemia using twelve adults. (132, 133). Participants consumed pancakes containing either blueberries, raspberries, or berry-free pancakes matched for 50g glycemic



carbohydrate. The pancakes contained 50g of raspberries or blueberries with accompanying 50g berries, resulting in a total of 100g of berries. No differences were observed in the glucose response after all three treatments were consumed. In the present study, participants were given 150g of blueberry purée, which is equal to 150g fresh blueberries, providing 39.1g glycemic carbohydrate along with white bread. The discrepancy between the results of both studies could be attributed to the differences in doses of blueberries and, respectively, dietary fiber and polyphenols available to cause the reduced blood glucose response within 30min which was not observed by Clegg et al (2011) (132).

The present study used a dose of 150g of blueberry purée similar to the study conducted by Törrönen et al. (2010) (134) in which plasma glucose concentrations were found to be significantly lower within 15 min and 30 min when berries were consumed in a form of blended purée served with 35g sucrose compared to the control (250ml water with 35g sucrose) (134). This confirms the pattern observed in the present study where blood glucose was found to be lower by 8.5% at 15 min, and by 6.5% at 30 min compared to the control ( $P < 0.05$ ). The HTD processing does not eliminate the blood glucose lowering effect observed for conventionally produced blueberry purée.

Total AUC was indicated to be a better indicator of physiological state since it is not entirely affected by the varying baseline unlike net AUC and incremental AUC (135). Summary calculations such as the AUC give a better indication of individual metabolic responses compared to the mean blood glucose concentration (136). Total AUC, net AUC, and incremental AUC for the postprandial glucose response upon consumption of the treatment were not significantly different over two hours, which agrees with findings from

Törrönen et al. (2010) and Clegg (2011) (133,134). However at 30min, total AUC, net AUC, and incremental AUC were significantly lower after consuming BB. The lack of the effect over 45-120 min could be due to the low fiber and polyphenols content in 150 g of BBs.

Overall, similar to Törrönen et al. (2010), the peak serum glucose was observed at 30 min after consumption of the control meal and at 45 min for the BB. Also, the control meal peaked at  $6.64 \pm 0.98$  mmol/L while the BB was at  $6.35 \pm 1.07$  mmol/L. This might be due to the major difference of fiber and polyphenol content of the HTD processed blueberry beverage. Usually, after consumption of carbohydrates, peak blood glucose concentration occurs at 30min. However, when consumed with high fiber and or bioactive containing fruits, the peak is delayed. Health benefits are hastened when fruits are in liquid since they require less time for digestion and absorption than when they are in their solid form as confirmed by existing evidence of their ability to reduce the rate of sucrose digestion and glucose absorption (7-9). The dietary fiber present in the blueberry inhibits  $\alpha$ -amylase activity by adhering to its binding sites on the starch molecules (52). As a result, the rate of starch hydrolysis to glucose is reduced, reducing the release of glucose into the blood. Adsorption of the fiber molecules to glucose also decreases diffusion of the glucose into the blood. Dietary fiber is also able to increase viscosity in the small intestines where significant digestion and absorption occurs. The higher viscosity retards the rate at which glucose diffuses (52).

Moreover, blueberries contain flavonols like quercetin and myricetin, ascorbic acid, hydroxybenzoic acids like gallic acids and protocatechuic acids, hydroxycinnamic acids like caffeic acids, ferulic acids and coumaric acid, anthocyanins, resveratrol, and pterostilbene

(104,105). The health benefits of these components are associated with their chemical structures. Proanthocyanidins attenuate activities of  $\alpha$ -glucosidase thereby reducing the rate at which carbohydrates are hydrolyzed into glucose (7), ameliorate the antioxidant environment in pancreatic  $\beta$ -cells, and reduce intestinal glucose absorption by inhibiting intestinal  $\alpha$ -glucosidase activity (113). These are likely reasons for the attenuated and delayed postprandial blood glucose response observed after consumption of the blueberry purée. Phenolic acids also inhibit glucose uptake in the intestines after hydrolysis (57). This further explains the difference in the change in mean blood glucose from the baseline level observed between the two treatments at 30min. Although the mean baseline blood glucose was not significantly different when participants arrived in the lab for the experimental session, the change in blood glucose at 15mins and 30mins was significantly higher after control meal was consumed compared to the blueberry treatment.

Overall there was no significant effect of sex ( $P = 0.19$ ) on the mean blood glucose concentrations although different trends were observed in males versus females. During the luteal phase of the menstrual cycle, glucose metabolism is impaired (137). Since no significant effect of sex was seen, scheduling of female participants during follicular phase was effective in ensuring no influence of hormones on glucose metabolism. In males, the peak blood glucose concentration for water treatment was sustained at 6.98 mmol/L from 30 min to 6.97 mmol/L at 45 min after which it reduced to 4.65 mmol/L at 120 min. On the other hand, BG peaked at 6.84 mmol/L at 45min and diminished to 4.65 mmol/L for males after consumption of the blueberry beverage. For females, the peak BG for both water and blueberry beverage occurred at 30minutes at 6.28 mmol/L and 6.18 mmol/L, respectively. Similarly, at 120 min the BG measured was 4.28 mmol/L for both treatments however with

different reduction patterns. BG was significantly higher at 15 min ( $P = 0.02$ ), and 30 min ( $P = 0.04$ ) in males after consuming the control meal. In females, no significant differences were observed in postprandial blood glucose concentration after both treatments. However, at 90mins, although not significant ( $P = 0.08$ ), female blood glucose was higher after the BB treatment. There is evidence suggesting that oral contraceptives affect glucose metabolism in a dose-response manner while others indicate otherwise (138,139). Some female participants were on oral contraceptives and that could have caused the difference in glucose response observed between males and females since hormonal changes from the menstrual cycle was controlled for (137, 140).

The HTD processing did not eliminate the ability of blueberries to reduce postprandial blood glucose.

## **6.2 Postprandial antioxidant status**

In this study there was no significant effect of treatment and time on the total antioxidant capacity within the 2-hour period as assessed by TEAC. Kay and Holub (2002) found a significant treatment effect ( $P = 0.001$ ) after providing 100g freeze-dried blueberries. The 100 g of freeze-dried blueberries (500-650g fresh fruit) might have a higher potency than 150 g purée. It is also likely that any possible effect of BB treatment may have been observed over a longer postprandial period since total anthocyanin concentration in blood increases with time. There was no significant difference observed in the present study between total AUC, incremental AUC and net AUC of the total antioxidant capacity. The difference in the outcome of both studies could be due to the length of blood (4 hours) sample

collection, the difference in processing technique, bioavailability, and concentration of beneficial polyphenols.

The TEAC assay is based on the ability of antioxidants present in plasma samples to suppress the production of the radical cation. The antioxidant capacity depends on how much antioxidant molecules there are. No significant treatment effect could mean that the antioxidants present in the treatment might have suppressed the free radicals from white bread leaving little or no molecules to suppress the ABTS radical cation within the 10min period of the assay. Therefore, the assay appears to be a contributing factor of the results attained. Such an effect was seen by Kay and Holub (2002) when ORAC assay indicated an increase from the baseline serum antioxidant capacity in the control and blueberry treatment groups while the TEAC assay indicated otherwise in the control group (10). Moreover, ferrylmyoglobin radicals might also be reduced by antioxidant molecules from treatment, hence underestimation of the total antioxidant capacity. In using ORAC, significant treatment and time effects were observed based on the increased serum antioxidant concentration ( $P = 0.04$ ) when blueberry powder was consumed. However, this was not seen in the current study. There was no significant difference in the Total Antioxidant Capacity (TAC) at each time point.

There was an effect of sex ( $P = 0.01$ ) but no significant treatment\*sex interaction ( $P = 0.31$ ). Within the 2-hour period, when males consumed treatments, TAC values were mostly higher for the control compared to females. The reverse was observed in females. Given that both males and females consumed about 150 g of BB, the effect of sex could be a result of reduced anthocyanin compounds available per kg body weight. Different mean weight

was observed across both sex groups. The mean weights for male and female participants were  $72.43 \pm 7.78$  kg and  $58.55 \pm 9.20$  kg, respectively. Mazza et al. (2002) provided 15 mg/kg of body weight anthocyanins to observe significant increase serum antioxidant status (120,141). This potentially explains the difference observed between sexes in the present study.

Antioxidant properties *in vitro* are mainly due to anthocyanins but not vitamins, fibers or antioxidant minerals (107). Antioxidants are able to donate electrons to highly reactive species to halt the chain reaction or detoxify free radicals (11). In addition, ascorbic acid was found to enhance the antioxidant capacity of blueberries by 10% by Barberis et al., (2015) while Borges et al., (2010) indicated that anthocyanins contributed about 84% (121,122). Mazza (2002) indicated that 0.002 - 0.003% of consumed anthocyanins were detected in the serum of participants (142). The anthocyanin content of BB was  $87.47 \pm 4.79$  mg/100 g, giving about 131.2 mg/150 g which is lower than 1.20 g provided by Mazza (2002). This could be a possible reason for the observations of no significant treatment or time effect in the current study.

### **6.3 Limitation**

The total antioxidant capacity of the treatments before consumption was not measured. Moreover, the level of polyphenols in blood upon consumption of the treatments were not measured either.

A longer postprandial testing period could have shown if these polyphenols have the ability to increase serum antioxidant status. The 2-hour period may not have been long enough for uptake and appearance of polyphenols in the serum.

### **6.4 Implication for Future Research**

Blueberries may have beneficial effects on health in general; the required amount of HTD processed blueberry purée for these beneficial effects on antioxidant status to be realized could be further studied. The effect of blueberry on postprandial antioxidant status could benefit from being studied over a longer period of about 4 hours. Determining the difference between conventionally processed purée and HTD processed purées would enhance understanding of the health benefits of HTD processed food products.

Further investigation into the level of oxidation that usually results from the accompanying meal and the amount of HTD processed blueberry purée that would have to be consumed to a sufficiently halt resulting free radicals might be helpful. It seems that a sufficient level of mg/kg of body weight anthocyanins must be attained to observe a significant increase in serum antioxidant status. It will be beneficial to determine a suitable dose to provide the required mg/kg of body weight anthocyanins.

## **7.1 Conclusion**

In healthy adults 19-35 years, hydrothermodynamic processed blueberry purée reduced and delayed the peak of blood glucose concentration over half an hour after consumption compared to a sweetened water of the same level of glycaemic carbohydrate when eaten with high glycaemic meal (white bread). The HTD processed blueberry beverage did not affect postprandial antioxidant status within two hours as measured by trolox-equivalent antioxidant capacity.

## **7.2 Perspectives**

To better understand the effect HTD-processed blueberries have on postprandial blood glucose control and antioxidant status in human adults, the beneficial components of interest (fiber and polyphenols) need to be assessed in the blood. The uptake and bioavailability of the bioactive components need to be determined.



### **7.3 Practical Application**

From the outcome this study, the HTD processed blueberry purée could be used as healthier alternative to sugar sweetened beverages. In 150g (141.5ml) of the purée, the dietary fiber and polyphenol reduced blood glucose response over half an hour. The HTD processed blueberry purée could be eaten during any eating event of the day, to help meet recommended daily fruit and vegetable servings based on Health Canada recommendations. Health Canada recommends 125ml as a serving of fruits and vegetables, which is slightly lower than the amount used in this study (141.5ml).

Blueberries are seasonal and might not be available in many areas when not in season. The HTD processed blueberry purée has an extended shelf life, therefore when raw blueberries are out of season it provides a good alternative.

Findings from this study further confirm the health benefits of consuming blueberries, particularly the benefits of the novel HTD processing method of producing whole purée containing seeds and skins. Since the purée is in the liquid state, the ability to reduce postprandial blood glucose response is attained faster. HTD processed blueberry purées could be added to the ever-growing list of healthy food innovations.

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## Appendices

### Appendix 1: Advertisement Poster



## **ATTN: ADULTS AGED 19-35**

We are conducting a research study to learn more about the health effects of a new processed whole berry beverage product on blood sugar control and inflammation.

REQUIREMENTS: Adults aged 19-35

Healthy, and not be taking medication

INVOLVES: screening with the information session  
and two 2.5 hour study sessions.

Participants will be asked to consume a beverage, and give blood samples  
over a course of 120 minutes.

To thank you for participation in our study:

You will receive \$30 for each study session

Plus \$5 per visit or bus tickets for travel reimbursement

Please contact us at: (902)-457-6378 or e-mail us:

[Appetite.study@msvu.ca](mailto:Appetite.study@msvu.ca)

## **Appendix 2: Telephone Screening Questionnaire**

### Telephone Screening Questionnaire Part 1 of 3

#### Methods

##### Purpose:

This questionnaire will be administered by the researcher for the purpose of recruitment of participants.

##### Methods:

The researcher will ask to speak with the indicated potential participant. If the researcher is unable to reach the participant, they will leave a message and try again the following day. If they do not hear back they will try one more time and if they are unsuccessful again they will remove the participant from the sample pool, assuming that the participant is not interested in the study.

Telephone Screening Questionnaire Part 2 of 3

Participant Identity

Name: \_\_\_\_\_ ID assigned: \_\_\_\_\_

Age: \_\_\_\_\_ years

Month and Year of Birth: \_\_\_\_\_

TO BE KEPT SEPARATELY



Telephone Screening Questionnaire: part 3 of 3

*Please print or circle the answer*

ID: \_\_\_\_\_

Age: \_\_\_\_\_

Height: \_\_\_\_\_ cm Weight: \_\_\_\_\_ kg

Have you lost or gained weight recently? Yes / No

Do you follow a special diet? Yes / No

Do you have any food allergies or food sensitivities? Yes / No

(If yes please explain: \_\_\_\_\_)

Health Problems? Yes / No (If yes please explain: \_\_\_\_\_  
\_\_\_\_\_)

Medications: Yes / No (If yes please explain: \_\_\_\_\_  
\_\_\_\_\_)

Do you have any learning difficulties/problems? Yes / No

(If yes please explain: \_\_\_\_\_)

Do you have any behavioral or emotional problems? Yes / No  
(If yes please explain: \_\_\_\_\_)

Include in study? Yes / No

Appointment scheduled for: (date and time)

\_\_\_\_\_

Investigator/Date screened:

\_\_\_\_\_

### Appendix 3 : Baseline Information Questionnaire

#### Baseline Information Questionnaire

(NOTE: After you are recruited for the study, you will be assigned an ID# which will be used on your forms and data throughout the study.)

AGE: \_\_\_\_\_ HEIGHT: \_\_\_\_\_ WEIGHT: \_\_\_\_\_ BMI: \_\_\_\_\_

Participation in Athletics/Exercise:

ACTIVITY	HOW OFTEN?	HOW LONG? (HOURS)

Do you usually eat breakfast?  YES  NO  
If YES, what do you usually eat? \_\_\_\_\_

Health Status:

Do you have diabetes?  YES  NO  
Do you have any other major disease or condition?  YES  NO  
If YES, please specify: \_\_\_\_\_

Are you taking any medications?  YES  NO  
If YES, please specify: \_\_\_\_\_

Do you have reactions to any foods?  YES  NO  
If YES, please specify: \_\_\_\_\_

Are you on a special diet?  YES  NO  
If YES, please specify: \_\_\_\_\_

Have you recently lost or gained weight?  YES  NO  
If YES, please specify: \_\_\_\_\_

Do you smoke?  YES  NO

How many alcoholic beverages do you consume per day? \_\_\_\_\_ Per week? \_\_\_\_\_

## Appendix 4: Menstrual Cycle Questionnaire

### Menstrual Cycle Questionnaire

ID: \_\_\_\_\_ Date: \_\_\_\_\_

1. My average cycle length is \_\_\_\_\_ days.
  2. Currently, for how many days do you typically experience menstrual flow each cycle?  
\_\_\_\_\_1 day    \_\_\_\_\_2 days    \_\_\_\_\_3 days    \_\_\_\_\_4 days    \_\_\_\_\_5 days    \_\_\_\_\_> 5+ days
  3. My periods are regular \_\_\_\_\_ Yes; \_\_\_\_\_ No (Schedule to be confirmed for each session)
  4. The date of my last period was \_\_\_\_\_ (day/month/year)
- 

#### *To be completed by Research Personnel:*

1<sup>st</sup> Session: \_\_\_\_\_ (day/month/year)

2<sup>nd</sup> Session: \_\_\_\_\_ (day/month/year)

## Appendix 5a: Consent Form for Participant



### Consent Form for Participant for Clinical Trial

#### Investigators:

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## INTRODUCTION

You are invited to take part in the research study named above. This form provides information about the study. Before you decide if you want to participate, it is important that you understand the purpose of the study, the risks and benefits, and what you will be asked to do. We will provide you with information before asking for your authorization to participate. We will keep you informed of any new information that might influence your willingness to continue participating. A member of the research team will be available to answer any questions you have. You may decide not to participate or you may withdraw from the study at any time. You do not have to take part; it is entirely voluntary.

*Why are the researchers doing the study?*

Whole berries, such as blueberries and cranberries, are well known for their health benefits. Traditional berry beverages use processing methods that remove many of the beneficial properties of whole berries, such as fiber and antioxidants. A new method of processing whole berries has been shown to retain many of these healthy properties. The researchers are hoping to gain an understanding of how these whole berry beverage products are accepted by adult consumers, and to later demonstrate the health benefits of these beverages.

We hope that the information from this study will encourage further development of whole berry food products while increasing consumption of berries in Canadians.

This experiment is being conducted through the Department of Applied Human Nutrition at Mount Saint Vincent University. You will be asked to attend one screening session and two experimental sessions conducted on the mornings and for a total of 3 visits to the Mount Saint Vincent University campus.

This study will not cost you anything. You will receive \$30 for each session you participate in.

We anticipate having about 26 participants enrolled in this study which is the part of a large research project that is financially supported by Agriculture and Agri-Food Canada and Canada Food Innovators Cluster Program, in partnership with PEI Berries. There are no conflict of interests between the investigators and the sponsor.

## PROCEDURE:

Screening:

You will be asked to complete a questionnaire to determine if you meet the eligibility criteria for the experiment.

Experimental sessions:

You will be asked to go to Mount Saint Vincent University (MSVU) for two individual morning sessions. These sessions will be held on weekends or holidays or in summer during vacations over three weeks.

We are asking that you arrive at MSVU at 9:00 am after completing an overnight fast. After arrival to the lab, you will be asked to complete a questionnaire about your recent food intake and if any medication was taken. You will then be asked to have a blood sample taken to measure your fasting blood sugar levels. After this, you will be asked to consume one of two different products, either a berry product or a sweetened water, both of which will be followed by two slices of white bread. You will then be asked to provide a further 6 blood samples (for a total of 7) over the next 2 hours, at 15, 30, 45, 60, 90, and 120 minutes, after which you will be provided with an area to rest. Snacks will also be provided once the study is completed.

All blood samples gathered will be done so by a trained Nurse or Phlebotomist, and all samples will be gathered using aseptic techniques in a hygienic environment. Each sample will be 8.5 ml, for a total of less than 60 ml, far less than that taken when donating blood.

Approximate Time and Activity Schedule for Each Experimental Session:

Time	Activity
9:00 am	You will arrive to the lab
9:05 – 9:20 am	Complete baseline questionnaires for recent food intake, appetite and physical comfort
9:20 am	Nurse or phlebotomist will collect fasting blood sample and your blood sugar level will be checked.
9:25 am	You will consume the beverage with white bread
9:35 – 11:25 am	Nurse or phlebotomist will be collecting your blood samples for two hours. During that time, the tiny tubing will remain in your vein so you will not be poked every time when blood needs to be drawn.
11:30 am	Experiment is complete. We will provide you with a snack and beverage.

CONFIDENTIALITY:

Your records will be kept confidential in a locked cabinet in the Department of Applied Human Nutrition and no disclosure of your personal information will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data from participants who have completed the study will be entered into Microsoft Excel files, available only to investigators. The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported. Each participant will have a coded file, also only available to the investigators. If you choose to withdraw from the study, all your data will be removed and all hard copies will be destroyed. All forms and printouts will be stored in the individual files and clearly labeled. All documents will be kept for a minimum of five years and then securely destroyed. No disclosure of personal information of participants will take place except where required by law, for example concerns of suspected child abuse.

#### RISKS:

There are very little risk related to this study. Our research team will make sure all foods served to you are fresh so you will have very little chance to experience food poisoning. Your blood samples will be taken carefully by experienced nurse or phlebotomist and therefore the risk of bruising or infection is minimal.

#### BENEFITS:

There are no direct benefits from participating in the study. However the information gained in the study might help to better understand how beverage products made via a new processing method using whole berries influence blood glucose and antioxidant level in human body. The data gathered from this study may support further studies in understanding the role of berries, and the role of processing, on risk factors for chronic health conditions associated with impaired glucose control and inflammation. Each participant will receive a copy of Canada's Food Guide along with a copy of "My Food Guide."

#### QUESTIONS AND FURTHER INFORMATION:

Participation is completely voluntary. Also, you have the option to stop participating, skip any step/question or withdraw from the study at any time without any penalty or concern. You will still receive the compensation for eat session you participated in, regardless of whether you fully complete the study.

If you have any questions or would like further information concerning this research project, please do not hesitate to call our Study Coordinator Mr. Andrew Hamilton at (902) 457-6378 and leave a message. We will call you back shortly.

**If you have questions about how this study is being conducted and wish to speak with someone who is not directly involved in the study, you may contact the Chair of the University Research Ethics Board (UREB) c/o MSVU Research and International Office, at 457-6350 or via e-mail at [research@msvu.ca](mailto:research@msvu.ca)**

We may want to contact you in future to provide information about our other projects you may be interested in and invite you to participate in these projects.

#### PARTICIPANTS RIGHTS:

Your signature on the form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree that you will participate as a subject. You are free to withdraw from the study at any time.

By signing below, you also indicate that you understand that for purposes of the research project, if you choose to withdraw from the study at any time, you may do so without any problems.

Upon completion of each study session, you will receive a \$30. You are aware that the researchers may publish the study results in scientific journals, keeping your identity confidential.

#### RESEARCH RESULTS:

If you wish, a summary of the study results can be provided. They will be available around one year after the end of the study. The ethical components of this research study have been reviewed by the University Research Ethics Board and found to be in compliance with Mount Saint Vincent University's Research Ethics Policy.



Consent Form for Participant for Clinical Trial

Participant ID: \_\_\_\_\_

**PARTICIPANT AUTHORIZATION:**

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks. I understand that I have the right to withdraw from the study at any time without any problems. I have received a copy of the Information and Authorization Form for future reference. I freely agree to participate in this research study.

Name of Participant: (Print) \_\_\_\_\_

Participant Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

If you would like to receive the summary of the results, please print your address, or preferred method of contact, below:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT

I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Name: (Print) \_\_\_\_\_ Signature: \_\_\_\_\_ Position: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

**Appendix 5b : Consent to be contacted for participation in future studies**

Consent to be contacted for Participation in Future Studies

Would you like to be contacted for future research? Yes\_\_\_\_, No\_\_\_\_\_.

Name of Participant: (Print) \_\_\_\_\_

Participant Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

If you would like to be contacted for future research, please print your address, or preferred method of contact, below:

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## Appendix 6: Health and Activity Questionnaire

### Health and Activity Questionnaire

1. Did you have a normal night's sleep last night?

Yes \_\_\_\_\_ No \_\_\_\_\_

2. How many hours of sleep did you have?

\_\_\_\_\_

3. What time did you go to bed last night?

\_\_\_\_\_

4. What time did you wake up this morning?

\_\_\_\_\_

5. Recount your activities since waking:

Time	Activity
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

6. Are you experiencing any feelings of illness or discomfort, other than those from hunger?

Today: Yes \_\_\_\_\_ No \_\_\_\_\_

Past 24 hours: Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, please describe briefly:

\_\_\_\_\_

\_\_\_\_\_

7. Are you under any unusual stress?

Exams/reports/work deadlines, personal, etc.

Today: Yes \_\_\_\_\_ No \_\_\_\_\_

Past 24 hours: Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, please describe briefly:

\_\_\_\_\_

\_\_\_\_\_

8. Have you been involved in any physical activity within the past 24 hours that is unusual to your normal routine?

Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, please describe briefly:

\_\_\_\_\_

\_\_\_\_\_

9. Have you had anything to eat or drink, other than water and provided breakfast, for the past 11-12 hours?

Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, please describe briefly:

\_\_\_\_\_

\_\_\_\_\_

## **Appendix 7: Statement from Researchers ensuring ongoing consent**

### Statement from Researchers Ensuring Ongoing Consent:

We thank you for your ongoing participation in our study, and want to make sure that you feel that you are aware of and understand the potential risks and benefits of this study, as we previously discussed. We want to remind you that it is your right to withdraw from the study at any time without any problems, as we indicated with you on your copy of the Information and Authorization form.

**Appendix 8: TCPS 2 training certificate**

