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GLYCEMIC INDEX OF BREAD: FIBER OR MINERALS? THIS IS THE QUESTION

by
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AN ABSTRACT OF THE THESIS OF

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Glycemic index (GI) of food is known to be positively associated with the development of several diseases including type II diabetes. Modest dietary changes from high to low GI foods have shown beneficial effects. These findings have been translated to recommendations for increased dietary intake of whole grains. Trials of added cereal fiber however, have failed to induce a protective effect and fiber has thus been proposed to be a marker of other components of whole grains that impart health advantages. Whole wheat grains are known to be a rich source of several minerals (phosphorus, potassium and magnesium) that play a role in glucose metabolism, and are depleted during the process of refinement. Thus, it is plausible to hypothesize that the benefits of whole grains previously ascribed to their fiber content are in fact due to these minerals. Therefore, the proposed research aims to restore and fortify white bread with these minerals and determine their resulting GI.

This is a single blind cross over design study. Twelve healthy male subjects were recruited, and asked to complete a total of four visits. On each visit, participants consumed in random order one of the 4 different types of bread: white bread (WB), whole grain bread (WG), restored bread (WB-R), and fortified bread (WB-F). Blood samples were collected at fasting, 15, 30, 45, 60, 90 and 120 minutes post meal ingestion. Serum glucose, insulin, GLP-1, GIP, triglycerides, phosphorus, magnesium, and potassium were measured. GI was measured according to the trapezoidal rule of geometry and area under the curve.

WB-R and WB-F significantly retained the lowest levels of glucose at 60 minutes onwards, while insulin levels remained unaltered and did not show any significance. Both WB-R and WB-F also maintained significantly lower triglyceride levels than the WB group. Serum phosphate levels were significantly higher in WB-R and WB-F. Similarly, serum potassium levels were significantly higher in WB-F at 120 minutes. Serum magnesium, GLP-1, and GIP did not show any statistical significance. Among bread groups, WB-F significantly maintained the lowest GI as compared to WB.

These findings have successfully identified the potential beneficial role of minerals, particularly phosphorus, magnesium, and potassium in improving glycemic index of white bread and overall glucose control in healthy male subjects. Results from this study are promising and may prove to be the backbone for future research toward the establishment of a recommended mineral: carbohydrate ratio.

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ABBREVIATIONS

%	percent
&	and
/	per
=	equal
β	beta
Δ	change
ºC	degrees Celsius
2,3 – DPG	2,3-diphosphoglycerate
AACC	American Association of Clinical Chemistry
ACE	angiotensin-converting-enzyme
ADA	American Dietetic Association
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AUB	American University of Beirut
AUBMC	American University of Beirut Medical Center
AUC	area under the curve
BMI	body mass index
cAMP	cyclic adenosine monophosphate
CHD	coronary heart disease
CRU	Clinical Research Unit
d	day
dl	deciliter

DPP4	dipeptidyl peptidase-4
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FGF-23	Fibroblast growth factor 23
g	gram
G-6-P	glucose-6-phosphate
GI	glycemic index
GIP	Glucose-dependent insulinotropic peptide
GIP-R	gastric inhibitory polypeptide receptor
GLP-1	Glucagon-Like Peptide 1
GLP-1R	glucagon-like peptide 1 receptor
GMP	Guanosine 5'-Monophosphate
GNP	Gross National Product
$H_2PO_4^{-4}$	dihydrogen phosphate ion
HOMA	Homeostasis Model Assessment
HPO_4^{-2}	hydrogen phosphate ion
IOM	Institute of Medicine
IRB	International Review Board
K	potassium
Kcal	calories
kg	killogram
L	liter
m^2	meter squared
mEq	milliequivalent

Mg	magnesium
mg	milligram
min	minutes
ml	milli
mmol	millimole
n	sample size
Na	sodium
OGTT	oral glucose tolerance test
P	phosphorus
pg	picogram
PKA	protein kinase A
pM	picometer
pmol	picomole
PO_4^{-3}	phosphate ion
PTH	parathyroid hormone
RDA	recommended daily allowance
RPM	revolutions per minute
SEM	standard error of the mean
UL	upper limit
WB	white bread
WB-F	white bread fortified
WB-R	white bread restored
WG	whole grain bread
WHO	World Health Organization
yrs	years

CHAPTER I

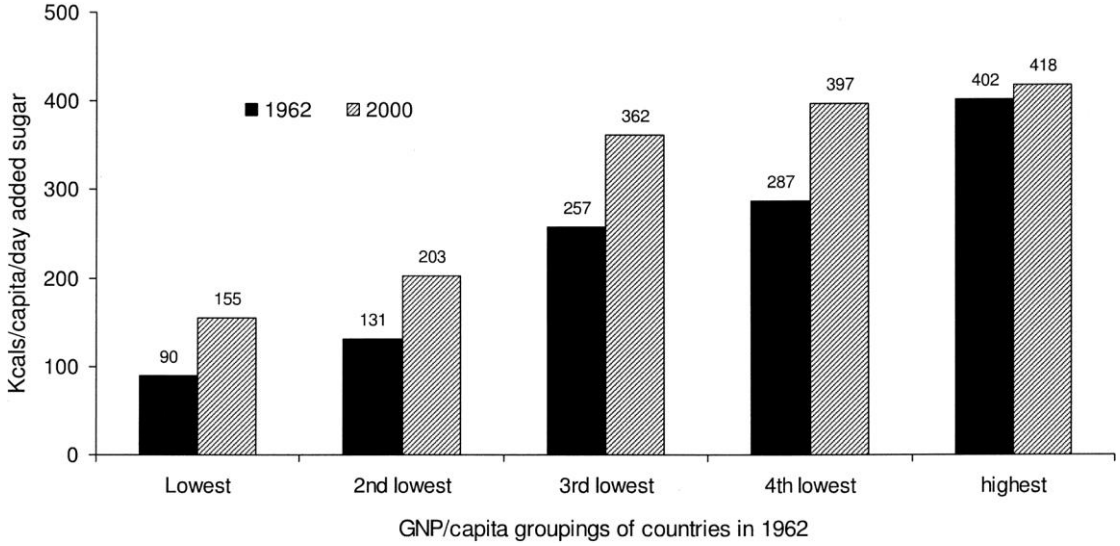
INTRODUCTION

Over the last decades, the world has been gradually witnessing several nutrition transitions, which have eventually caused significant changes in dietary habits. This shift in dietary consumption revolves mainly around the transition from traditional diets rich in complex carbohydrates, whole grains, cereals, fruits, vegetables, and legumes to diets high in refined cereal commodities, refined edible oils, and artificially sweetened foods mainly composed of simple sugars and refined carbohydrates. For instance, individual adults in China consuming more than 30% of energy intake from fat, increased from 15% to 44% between 1989 and 2000 (Popkin, 2006).

In the United States, calorically sweetened beverages account for more than 50% of the increase in added caloric sweeteners in the past several decades (Popkin, 2006). Consumption of sodas and other caloric beverages represented 21% of overall caloric intake in Mexico from 1996 to 2002 (Popkins et al., 2011). Figure 1 illustrates the changes in the per capita gross national product (GNP) and caloric sweetener intake between the years 1962 and 2000. It is well known by now that eating trends and patterns evolve over time as a result of several factors and merged interactions that tend to shape dietary habits, such as wages, prices, traditions, norms, beliefs, social factors, but most importantly globalization. Processes of globalization and economic advancement have had a vast effect on food markets, thereby making these energy-dense products affordable, inexpensive, easier to produce, and above all

palatable; all of which are channeled to cause an exponential increase in the demand of these products. The ongoing regional and global eating trends have led to increased rates of obesity -the worldwide epidemic-, and many other adverse health challenges, including components of the metabolic syndrome (impaired glucose tolerance, dyslipidemia, and hypertension). To be more specific, these transitions have led to increased consumption of electrolyte-free commodities (phosphorus, potassium, and magnesium) such as sweeteners, refined oils, and refined cereals commodities; all of which contain negligible amounts of the above minerals.

Figure 1: Changes in the per capita gross national product (GNP) and sweetener intake



Popkin, B. (2006). Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. *The American Journal of Clinical Nutrition*.

The high prevalence of obesity and metabolic syndrome among those consuming high amounts of electrolyte free commodities (particularly phosphorus, potassium, and magnesium), may associate the role of these minerals in the onset and development of metabolic syndrome. Therefore, high consumption of these electrolyte-free refined commodities is thought to compromise their postprandial serum status. Metabolically, such a compromise can induce several metabolic conditions that favor the development of the different components of the metabolic syndrome, one of which is impaired glucose tolerance. In light of that, it is also worth noting that grain refinement extracts the outer layer of the grain and this process is associated with significant losses of the fiber and mineral content. Refinement tends to make cereals more palatable for most people and the intake of refined cereals has considerably increased over the last few decades. Additionally, refined cereals such as white bread, or white rice, are known to promptly raise blood sugar and therefore are considered to have high a glycemic index (a numerical classification carried out on carbohydrate-containing foods to test their effect on blood sugar). Given the fact that cereals contribute to more than 50% of total energy intake in most countries (FAO, 2014) the transition from coarse cereals to refined cereals would not only lead to substantial reductions in the intake of minerals, but may also eventually lead to an impaired glycemic control due to their relatively high glycemic indices.

Glycemic index is known to be directly associated with the onset and development of many diseases including type II diabetes, metabolic syndrome, cardiovascular diseases, and age-related macular degeneration (Augustin et al., 2002). Hence, dietary guidelines recommend consumption of low GI diets to promote health and lessen incidences of many diseases, particularly in those at increased risk of diabetes and ageing populations (Nishida &

Nocito, 2007). Low glycemic index diets have been reported to improve both lipidemic and glycemic profiles (Goff et al., 2013) and thus an inverse relationship has been reported between increased intake of whole grains and the risk of the different components of metabolic syndrome (Ye et al., 2012). However, trials of added cereal fiber have failed to induce any protective effect, and fiber has thus been proposed to be a confounding marker of other components of whole grains that impart health advantages (Jenkins et al., 2002), and such an effect may be partially explained by the mineral content of whole grains. Such findings may further explain several paradoxical experimental observations; for instance, the high content of these minerals in dairy products may have contributed to the observed inverse association between dairy product intake and metabolic syndrome (Lutsey et al., 2008), especially given that calcium alone failed to explain such an association (Yanovski et al 2009). Thus, it is reasonable to hypothesize that the benefits of whole grains previously ascribed to their fiber content may in fact be due to these minerals, more specifically phosphorus, potassium and magnesium. In line with this, these minerals have been shown and proven to exhibit beneficial effects on both lipidemic and glycemic profiles (Lippi et al., 2009; Haap et al., 2006; Belin & He, 2007; He & MacGregor, 2008).

As for implementation, the numerous beneficial effects of whole grains are widely known, yet the adoption of diets rich in whole grains still faces resistance. This may be somewhat due to the low palatability of unrefined cereal products. With much evidence that most people prefer the taste and color of white bread, it is therefore of vital need to develop palatable cereals yet with low glycemic index. White flour is the basis for staple foods in both developed and developing countries and contributes extensively to high glycemic index diets. Thereby, modifying the glycemic index of white flour, while maintaining its palatability,

would be a practical approach to reduce the glycemic index of diets and risk of metabolic syndrome among those who consume it. Modifying glycemic index of white flour via the addition of minerals is proposed to be promising in light of recent findings.

In humans, and under normal conditions, energy metabolism is known to fluctuate diurnally, since meal ingestion causes a shift to carbohydrate metabolism, and an increase in both energy expenditure and carbohydrate oxidation (Cox et al., 2012). Meal ingestion leads to an increase in cellular uptake and utilization of glucose and minerals (mainly phosphorus, potassium, magnesium), as a result of increased insulin secretion and demand for metabolic processes, for instance cellular phosphorylation. Therefore, plasma status of these minerals depends not only on the mineral content of the meal, but also on insulin secretion, which is in turn related to the type of ingested carbohydrate. Previous studies have shown that ingestion of pure glucose is known to be associated with a decrease in postprandial plasma concentrations of these minerals (Khattab et al., 2011) and their inclusion in a meal was reported to ameliorate this postprandial decrease (Khattab et al., 2011; Kishimoto et al., 2010). Hence, what remains to be elucidated is whether these minerals can affect postprandial glucose and insulin metabolism; only then it is reasonable to postulate that the glycemic index of food or postprandial glucose status would be affected by both the type of carbohydrate and mineral content of the food.

This paper aims at determining the glycemic index of white flour after it has been supplemented with phosphorus, potassium, and magnesium; in an attempt to reveal whether the benefits of whole grains previously ascribed to their fiber content are in fact confounding the favorable role of these minerals. In this study, white flour supplementation was achieved via both restoration and fortification. Restoration is the supplementation of white flour with minerals to attain back their original levels (prior to processing and milling). Fortification on the other hand is the addition of minerals to a level that is 100% higher than their original content. Boosting the status of these minerals in food would ameliorate any metabolic change related to contemporary eating patterns and high intake of electrolyte-free commodities. This can only be accomplished by fortification of white flour and eventually allowing for the establishment of an acceptable carbohydrate to mineral ratio comparable to that of carbohydrate to thiamin.

CHAPTER II

LITERATURE REVIEW

A. The Glycemic Index

All types of dietary carbohydrates whether starch or table sugar, share a common physiological property: their ability to raise blood glucose concentrations postprandially. Glycemic index (GI) is a numerical system used for the classification of carbohydrate-containing foods according to their respective glycemic response (Ludwig, 2002). It is defined as the incremental increase in the area under the two-hour glucose response curve following the consumption of a standard amount of carbohydrate (from a test food) as compared to that of a reference food usually glucose or white bread (Ludwig, 2002). Glycemic index uses a spectrum ranging from 0 to 100, whereby higher values are attributed to foods that induce the quickest increase in blood sugar. Pure glucose is usually considered a reference point, and is given a Glycemic Index of 100 (Jamurtas et al., 2013). Foods composed of simple carbohydrates (monosaccharides, disaccharides, oligosaccharides) undergo rapid digestion and absorption, which consequently results in a rapid release of glucose into bloodstream, and thereby have a high GI. Inversely, foods composed of complex carbohydrates (starch, fiber) exhibit slower rates of digestion and absorption, release glucose more gradually into bloodstream, and are thus classified as low GI foods. Although the glycemic index of a certain food is primarily predicted by the nature of its carbohydrate content, yet other factors may

also affect digestibility and insulin secretion such as pH, cooking, processing, and co-ingestion of other food components (fiber, fat, protein) (Jamurtas et al., 2013).

1. Glycemic Index and Metabolic Responses

Postprandial glucose levels and correspondig insulinogenic responses are mainly determined by the carbohydrate content of a meal. In normal situations, insulin stimulates uptake of glucose by the liver and peripheral tissues, thus stimulating glycogenesis, and simultaneously suppresses gluconeogenesis and glycogenolysis (Jamurtas et al., 2013). Upon ingestion of high-GI foods, a sharp exaggerated peak in postprandial glucose levels and hence in insulin levels will have to occur (Jamurtas et al., 2013). This is generally escorted with decline in glucagon secretion and elevated concentrations of the gut hormones (incretins), such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which synergistically stimulate insulin secretion from pancreatic beta cells and restrains glucagon release from pancreatic alpha cells (Ludwig, 2002). The resulting elevation of insulin levels will not only cause an uninterrupted uptake of nutrients, but also their suppressed mobilization from tissues, thereby amplifying the anabolic response to eating (Ludwig, 2002). Sharp peaks in insulin levels, often lead to remarkable fluctuations in blood glucose levels, as it is usually followed by sharp declines in glucose levels followed by episodes of hypoglycemia and hyperphagia (Ludwig, 2002). Moreover, habitual consumption of high GI foods may eventually lead to high 24-hour blood glucose and insulin levels, as well as higher glycosylated hemoglobin concentrations (Ludwig, 2002). On the other hand, low GI foods are known to delay glucose digestion and absorption, hence avoiding an exaggerated rise in glucose and insulin levels and subsequent postprandial hypoglycemia, therefore

improving satiety and overall glycemic control (Augustin et al., 2002). This ability of foods to physiologically stimulate postprandial glucose and insulin levels is directly related to the prevention and management of obesity, hyperlipidemia, type II diabetes, and other components of the metabolic syndrome (Nimptsch et al., 2011).

2. Classification of Foods according to their Glycemic Indices:

Typically, simple sugars and refined starchy foods tend to have a higher GI than non-starchy vegetables, fruits, and legumes, which are considered to be low GI foods. Fats and meats do not have any glycemic index rating due to the fact that they do not contain any carbohydrates. It is worth noting nevertheless, that co-ingestion of fat or protein along with a carbohydrate-containing meal decreases the glycemic index of individual foods somewhat, however it does not alter their hierarchical categorization with respect to glycemic index (Estrich et al., 1967). Low GI foods have a glycemic index rating of 55 or less, medium GI foods between 56 and 69, and high GI foods have a glycemic index rating of 70 or more (ADA, 2013). Table 1 lists the glycemic indices of some common foods, in reference to white bread with a set GI of 100.

Table 1: Glycemic indices of some common foods

Food	GI_{wb}
Sucrose	92
Glucose	138
Fructose	32
Honey	104
Milk	39
Beans	40–60
Lentils	30–40
Pasta	50–70
Pizza	86
Cornmeal/cornflakes	100–120
White bread	100
Pumpernickel	58
Potatoes	120
Banana, ripe	85
Banana, underripe	43
Oranges	62
Grapefruit	36
Cherries	32
Tomatoes	13

GI_{wb}, standard food: white bread.

Augustin, L., Franceschi, S., Jenkins, D., Kendall, C., & La Vecchia, C. (2002). Glycemic index in chronic disease: a review. *European Journal of Clinical Nutrition.*

3. What affects glycemic index?

As mentioned earlier, although the GI of a certain food is mainly predicted by the nature of its carbohydrate content, yet other factors may also affect digestibility and insulin secretion such as pH, cooking, processing, and co-ingestion of other food components (fiber, fat, protein) (Jamurtas et al., 2013). Table 2 lists various common factors that may influence GI. For instance, fat and/or fiber content of a food may lower the GI. Ripeness and storage time on the other hand, tend to increase the GI of a food (ADA, 2013). Likewise, processing adds to the GI of a certain food (a whole fruit has a lower GI than its juice) (ADA, 2013).

Cooking methods also tend to play an important role in determining the GI of a food; the longer the food is cooked the higher its GI.

Table 2: Factors that may influence glycemic response and glycemic index

Factors that affect GI	*Factors that decrease GI*	*Factors that increase GI*
Nature of starch	↑ Amylose/amylopectin	↓ Amylose/amylopectin
Nature of monosaccharide components	Fructose	Glucose
	Galactose	
Viscous fiber	↑ Guar	↓ Guar
	↑ β-glucan	↓ β-glucan
Cooking/food processing	Parboiling	Extruding
	Cold extrusion	Flaking
		Popping
Particle size	Large particles	Grinding (small particles)
Ripeness and food storage	Unripeness	Ripeness
	Cooling	
α-Amylase inhibitors	↑ Lectins	↓ Lectins
	↑ Phytates	↓ Phytates
Nutrient-starch interactions	↑ Protein	↓ Protein
	↑ Fat	↓ Fat

↑ = high levels.
↓ = low levels.

Augustin, L., Franceschi, S., Jenkins, D., Kendall, C., & La Vecchia, C. (2002). Glycemic index in chronic disease: a review. *European Journal of Clinical Nutrition.*

4. Determination of Glycemic Index

Glycemic index is the incremental area under the 2-hour blood glucose response curve following a test food, compared to an equivalent carbohydrate amount of a reference food (either glucose or white bread) consumed by the same subject (Wolever et al., 1991). Figure 2 shows the area under the curve (AUC) of a high versus low GI foods (Nayak et al., 2014). The World Health Organization (WHO) has set GI methodology guidelines for categorization of food items according to their respective GI values (Nayak et al., 2014). Following an overnight fast of 10 to 12 hours, blood samples are withdrawn from subjects at 7 time intervals: fasting and at 15, 30, 45, 60, 90, and 120 minutes after the start of the test meal, the

area under the curve is then calculated according to the trapezoidal rule in geometry (Brouns et al., 2005). Next, the GI rating (%) of a test food is calculated by dividing the AUC for the test food by the AUC for the reference food, and multiplying by 100 (Wolever et al., 1991). The average GI value is then calculated from data collected in a minimum of 10 human subjects (Nayak et al., 2014).

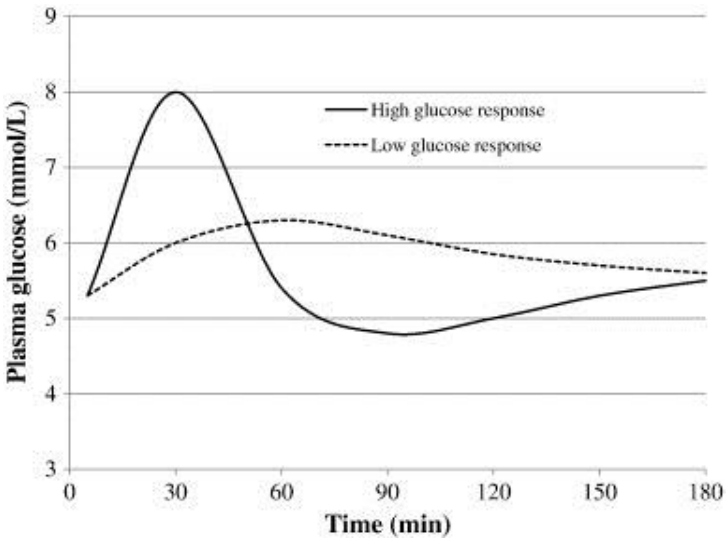


Figure 2: Incremental blood glucose area under the curve following a test food

Nayak, B., Berrios, D., Tang, J., & others,. (2014). Impact of food processing on the glycemic index (GI) of potato products. *Food Research International*.

B. Nutrition Transition and Whole Grain Consumption

Grains have always been considered to be the essential and indispensable food source for humans. The major cereal grains consist of wheat, rice, and corn, whereas the minor grains consist of oats, rye, barley, triticale, sorghum, and millet (Potter, 1996). During the past years, health-promoting effects of whole-grain foods have been widely investigated, and prospective cohort studies have associated a high consumption of whole-grains with reduced risk of cardiovascular disease, cancer, obesity, type II diabetes, and other chronic diseases (Pol et al., 2013). Improvement in the methods of processing and milling of grains however, widened the doors for large scale segregation and isolation of the bran and germ, thereby resulting in production of refined flour, which consists of the endosperm only. Refined flour have received worldwide acceptance and recognition, since it was used to produce baked goods that are more palatable, softer in texture, and with extended freshness.

1. Mineral Enrichment

In the early 1940's, attempts to enrich refined flour with B vitamins (thiamin, riboflavin, and niacin) and the mineral iron started (IOM, 2013). The term 'enrichment' is referred to the addition of micronutrients (vitamins and/or minerals) to restore nutrients back to their original levels – prior to milling and processing (FDA, 1996). It is widely known that vitamins and minerals are known to affect energy metabolism; for instance thiamin, a B vitamin in which its deficiency may cause Beriberi. This has led to the establishment of an acceptable carbohydrate and energy: thiamin ratio (0.4mg of thiamin/1000 kcal) and many countries are fortifying their white wheat flour with thiamin to sustain an acceptable ratio

(IOM, 1998). Although several minerals including phosphorus, potassium, and magnesium are known to affect energy and carbohydrate metabolism, however no clear ratio has been put in place yet.

2. Anatomy of a Whole Grain Kernel

A whole grain encloses the whole seed, also known as the kernel, of a plant. The seed or kernel itself is composed of three main elements: the bran, the germ, and the endosperm. The bran is the outer layer of the kernel that protects the other two components of the kernel. The bran is highly rich in antioxidants, fiber, B vitamins, iron, zinc, copper, magnesium, phytic acid, and other phytochemicals, all of which are proposed to be the basis of the health-promoting effects of whole grains (Slavin et al., 1999). The germ, which serves as the kernel's embryo, includes antioxidants, B vitamins, vitamin E, as well as other minerals and phytochemicals. Finally, the endosperm which comprises the largest portion of the grain, serves as a source of energy to the kernel. The endosperm is mainly composed of starchy carbohydrates, small amount of proteins, and negligible amounts of vitamins and minerals (Slavin et al., 1999). The process of conventional milling and grain refinement to produce white flour is based on the removal of the kernel's bran and germ, thereby leading to significant losses of fiber, B vitamins, minerals, and phytochemicals. The compositional differences between whole and refined grains are listed in Table 3. As the production of white flour requires the milling of the wheat grain and extraction of a proportion of the grains, the level of 80% extraction is the most-commonly used in the production of white flour in the market. Table 4 illustrates the substantial loss of the minerals phosphorus, potassium, and magnesium.

Table 3: Compositional differences between whole and refined wheat

Component	Whole wheat	Refined wheat
Bran (%)	14	<0.1
Germ (%)	2.5	<0.1
Total dietary fiber (%)	13	3
Insoluble dietary fiber (%)	11.5	1.9
Soluble dietary fiber (%)	1.1	1.0
Protein (%)	14	14
Fat (%)	2.7	1.4
Starch and sugar (%)	70	83
Total minerals (%)	1.8	0.6
Selected minerals		
Zinc (μg/g)	29	8
Iron (μg/g)	35	13
Selenium (μg/g)	0.06	0.02
Selected vitamins		
Vitamin B-6 (mg/g)	7.5	1.4
Folic acid (mg/g)	0.57	0.11
Phenolic compounds		
Ferulic acid (mg^{-2}/g)	5	0.4
β-tocotrientol (μg/g)	32.8	5.7
Phytate phosphorus (mg/g)	2.9	0.1

Slavin, J., Martini, M., Jacobs, D., & Marquart, L. (1999). Plausible mechanisms for the protectiveness of whole grains. *The American Journal Of Clinical Nutrition*, *70*(3)

Table 4: Phosphorus, potassium, and magnesium content in whole and refined flour

Mineral content	**Wheat flour (white)**	**Wheat flour (whole grain)**	**Loss (%)**
Phosphorus(mg/100g)	108	346	69
Potassium (mg/100g)	107	408	74
Magnesium(mg/100g)	22	138	84

Agricultural Research Service - United States Department of Agriculture USDAA National Nutrient Database for Standard Reference Release 26

C. Phosphorus: The Mineral

The mineral phosphorus is known to be the second most abundant essential mineral in the human body after calcium (Raina et al., 2012). Overall body stores of this mineral are estimated to be around 700 grams, of which 85% is localized in the bones and teeth in the form of hydroxyapatite (Wagner, 2007). Of the remainder, approximately 14% is found intracellularly and only 1% extracellularly, with an intracellular to extracellular ratio of 100:1 (Moe & Daoud, 2014; Gaasbeek & Meinders, 2005). Of this 1% of extracellular phosphorus, 70% is organically bound to phospholipids, and only 30% is inorganic; of the latter, 85% is either bound to sodium, magnesium, or calcium, or is in the ionized forms HPO_4^{2-}, H_2PO^{4-}, and PO_4^{3-}; the remaining 15% is bound to proteins (Moe & Daoud, 2014). Consequently, only 15% of extracellular phosphorus circulates freely in serum and plasma, and this (inorganic extracellular phosphate) is the fraction that is clinically measured (Moe & Daoud, 2014). Thereby, fluctuation in serum phosphate levels does not cardinally reflect total body stores of phosphorus (Raina et al., 2012). On the other hand, only 5% of the intracellular phosphate exists in the inorganic form, and 95% exists organically in the form of ATP (the body's major reservoir of biochemical energy), ADP, creatine phosphate, and nicotinamide adenine dinucleotide (Gasbeek and Meinders, 2005). An additional form of intracellular phosphate is 2,3 –diphosphoglycerate (2,3-DPG), an essential compound that mediates oxygen dissociation from hemoglobin in erythrocytesm ensuring its delivery to the tissues (Gasbeek and Meinders, 2005).

1. Phosphorus metabolism

A typical diet provides around 20 mg/kg of phosphorus per day, of which 60 to 70% gets absorbed in the small intestine via both paracellular diffusion and saturable carrier-mediated active transport system (Raina et al., 2012). High intake of dietary phosphorus is known to result in an increase in intestinal phosphate absorption with little evidence of an upper limit or saturation of absorption process (Wagner, 2007). Upon absorption, phosphate enters circulation and is then trapped up by bones, teeth, and other soft tissues; around 3 mg/kg/day of phosphorus is exchanged between mineralized bones and the extracellular fluid (Raina et al., 2012). Lastly, renal handling of phosphorus (excretion and reabsorption) is the utmost contributor to general phosphate homeostasis, whereby 85% of the filtered phosphate gets actively reabsorbed (Dimeglio & Imel, 2013).

2. Physiological functions of phosphorus

Apart from its crucial role in energy production and metabolism mentioned previously, phosphorus is considered to be an indispensible factor in virtually every biochemical reaction taking place, as well as other physiological functions, including bone mineralization, maintenance of cell integrity and structure (cell membrane phospholipids), phosphorylation of key enzymes and compounds , cell signaling (cyclic AMP and cyclic GMP), platelet aggregation, building block for nucleic acids, and maintenance of acid–base homeostasis (Raina et al., 2012; Moe & Daoud, 2014).

3. Dietary Phosphorus

The recommended daily allowance (RDA) of phosphorus for adult males and females is 700 mg/day, with a tolerable upper intake level (UL) of 4 g/d (Dimeglio & Imel, 2013). The average American diet contains approximately 1000 to 1400 mg of phosphorus per day (Moe & Daoud, 2014). Phosphorus is widely spread in food, with the richest sources known to be: high-protein foods such as meats, dairy products, poultry, fish and eggs, in addition to legumes, nuts, whole grains, and processed foods (with added phosphate for preservatives) (Dimeglio & Imel, 2013). For that, dietary induced phosphate deficiency is often uncommon, unless in the context of starvation or severe malnutrition.

4. Hypophosphatemia and Hyperphosphatemia

Normal serum phosphate concentrations are tightly controlled and range between 2.5 and 4.5 mg/dl (Moe & Daoud, 2014). This homeostasis is achieved through an interplay of several endocrine factors, which include: parathyroid hormone (PTH), vitamin D (1,25-dihydroxyvitamin D3), and fibroblast growth factor 23 (FGF-23) (Raina et al., 2012; Kuroo, 2010). Hyperphosphatemia is defined as plasma inorganic phosphate concentration reaches to levels greater than 5 mg/dl (Caudarella et al., 2006). This condition usually causes parathyroid gland hyperplasia and in turn increased serum parathyroid hormone levels (Kuhlmann, 2006). In addition, increased levels of inorganic phosphate may increase risk of coronary calcification and cardiac dysfunction (Kuhlmann, 2006). Hyperphosphatemia is mainly caused by decreased renal excretion (i.e. renal failure, hypoparathyroidism), metabolic acidosis, and erythrocyte hemolysis (Dibartola & Willard, 2012).

Hypophosphatemia on the other hand, occurs when plasma inorganic phosphate concentration is less than 2.0 mg/dl. It can occur when total body stores are normal; yet the most severe physiological and cellular damage tends to happen during phases of concurrent phosphate depletion (Levine & Kleeman, 1994). Consequences of hypophosphatemia include decreased red blood cells concentrations of ATP, increased red blood cells fragility and thus hemolysis, decreased red blood cells concentrations of 2,3-DPG, thus decreasing oxygen delivery to tissues, and platelet abnormalities (Craddock et al., 1974). Symptoms of hypophosphatemia unclude muscle weakness, fatigue, anorexia, and paralytic ileus (Knochel, 1977). Reduced serum phosphate levels may as well impair glucose uptake by central nervous system and thus decreased ATP production, resulting in metabolic encephalopathy and seizures (Levine & Kleeman, 1994). Causes of hypophosphatemia include misdistribution and rapid shift of phosphate from extracellular to intracellular fluid (i.e. refeeding syndrome, treatment of diabetic ketoacidosis), increased renal loss (i.e. hyperparathyroidism, renal tubular disorders), and decreased intake (i.e. phosphate binders, starvation) (Levine & Kleeman, 1994; Dibartola & Willard, 2012).

5. Phosphorus and glycemic control

Phosphorus is an essential mineral, known to be involved in several aerobic and anaerobic metabolic reactions especially those of energy and glucose metabolism. Inorganic phosphate takes part in the production of ATP in both glycolysis and oxidative phoshphorylation - Krebs cycle (Ditzel & Lervang, 2010). On the other hand, it also plays an essential role in carbohydrate metabolism via its phosphorylation/dephosphorylation means. The first step in glucose metabolism is its transport into cells where it undergoes rapid

phosphorylation to become glucose 6-phosphate (G-6-P) via the action of hexokinases, thus trapping the glucose within the cell (Bouche et al., 2004). This step is the most essential step in glycolysis, without which glucose wouldn't be transported into cells. Inorganic phosphate is also a substrate for the enzyme involved in glycolysis, glyceraldehyde-3-phosphate dehydrogenase (Ditzel & Lervang, 2010). Because inorganic phosphate is necessary in fueling glycolysis, low plasma concentrations may delay phosphorylation of carbohydrates and thereby glycolysis. Hence, chronic phosphate deprivation may impair glucose transport, carbohydrate metabolism, and eventually energy production (Kalaitzidis et al., 2005).

In several recent studies, hypophosphatemia was found to be significantly associated with impaired glucose tolerance and insulin sensitivity (Haglin, 2001; Lippi et al., 2009; Haap et al., 2006). Likewise, postprandial low serum phosphate levels were reported to be associated with elevated 2 hours blood glucose levels and reduced insulin sensitivity (Friedman, 2007). In the postprandial status, studies have found that plasma concentration of inorganic phosphate declines immediately upon the release of insulin, which in turn allows for the entry of plasma glucose and phosphate into the insulin-sensitive tissues for the purpose of cellular phosphorylation; thereby drastically decreasing plasma Pi (Ditzel & Lervang, 2010). In light of these findings, a recent study has revealed that the addition of 500 mg phosphorus to an oral glucose tolerance test (OGTT) was able to prevent the reduction in both total and inorganic phosphates (Khattab et al., 2011). At the same time, phosphate addition was able to reduce both insulin and HOMA at 60 minutes (Khattab et al., 2011). Thus, phosphate addition significantly improved insulin sensitivity after an oral glucose load (Khattab et al., 2011). The relation between inorganic phosphate and insulin sensitivity has been also recognized in

patients diagnosed with primary hyperparathyroidism. Hypophosphatemia secondary to hyperparathyroidism was thought to be the main reason behind the impaired glucose metabolism and decreased responsiveness to insulin seen in those patients (DeFronzo & Lang, 1980). Furthermore, researchers demonstrated that phosphate supplementation among insulin insensitive hypophosphatemic patients markedly enhanced insulin sensitivity and glucose tolerance (Wittmann & Nagy, 1997). Besides, studies performed on healthy subjects also revealed a positive relationship between decreased serum phosphate concentrations and insulin insensitivity and high postprandial blood glucose levels, independent of pancreatic insulin secretion (Haap et al., 2006). This indeed implies that it is insulin sensitivity and not insulin secretion that is mainly affected by low serum phosphate levels - even in healthy non-diabetic subjects (Haap et al., 2006).

D. Magnesium: The Mineral

The mineral magnesium is known to be the second most abundant intracellular cation, and is considered an indispensable cofactor in more than 300 enzymatic reactions (Touyz, 2004). Magnesium homeostasis is mainly dependent on the electrolyte's intestinal absorption (with an absorption rate between 30% and 50%), skeletal, cardiac, and smooth muscles distribution, and last of all on renal excretion rates (Belin & He, 2007). In the body, magnesium is contained in three main compartments: plasma and extracellular fluid (1%), intracellular fluid (34%), and in bones (65%) (Gunther, 2006). Serum magnesium concentration is tightly regulated at a narrow range of 1.5 to 2.5 mg/dl (Chubanov et al., 2005) of which 70-80% exists in the biologically active free form, and 20-30% coupled to

circulating proteins or complexed with anions, such as phosphate, or bicarbonate (Saris et al., 2000).

1. Physiological Functions of Magnesium

Magnesium exhibits a crucial role at both the cellular and biochemical levels. It takes part in several biological reactions essential for cell survival and normal functioning, including energy metabolism and production (ATP stabilization), nucleic acid and protein synthesis, maintenance of membrane integrity and stability, cytoskeletal function, ion homeostasis and cell cycle progression (Saris et al., 2000). Magnesium is also involved in nerve transmission, bone metabolism, cardiac excitability, and muscular contraction (Rude & Shils, 2005; Chubanov et al., 2005). Additionally, many studies have recently highlighted the effectiveness of magnesium in the therapeutic management and treatment of many diseases, including eclampsia, preeclampsia, hypertension, arrhythmia, asthma, and migraine (Guerrera et al., 2009).

2. Dietary Magnesium

The recommended daily allowance (RDA) of magnesium is 420 mg/day for men and 320 mg/day for women (Saris et al., 2000). In light of all the current food processing and altered eating behaviors, studies have found that around 75% of American citizens fail to meet the RDA (Alaimo et al., 1994). The mineral magnesium is widely distributed in food, including both animal and plant. Nuts such as almonds and cashews, green leafy vegetables such as parsley and spinach, legumes, seeds, and whole grains are known to be rich sources of

magnesium (Rude, 2012). Similar to other minerals, processes of grain refinement lead to substantial losses in magnesium content, upon removal of nutrient-rich bran and germ.

3. Hypomagnesemia and Hypermagnesemia

Symptomatic magnesium deficiency resulting from poor consumption of magnesium is usually rare, since the kidneys tend to by default decrease magnesium urinary excretion, as a regulatory response (Rude, 2012). Hypomagnesemia refers to a condition whereby serum magnesium concentration levels below 1.5 mg/dl (Saris et al., 2000). It may occur as a result of many factors such as starvation and habitual low intakes of magnesium, alcoholism, altered intestinal absorption, increased urinary excretion (i.e. uncontrolled diabetes mellitus), and the use of certain medications that may interfere in magnesium homeostasis (Touyz, 2004). Signs and symptoms of this medical condition include muscle weakness, spasms, loss of appetite, depression, and abnormal heart rhythms (IOM, 1997). Similarly, renal regulation prevents magnesium serum accumulation, and therefore as a regulatory response, kidneys tend to eliminate excess magnesium in the urine (Musso, 2009). Although magnesium toxicity from dietary intake is very unlikely, yet large doses of magnesium from supplements or medications (such as laxatives and antacids) may occur and can in turn lead to severe episodes of diarrhea, nausea, vomiting, and abdominal cramping (IOM, 1997). High doses of magnesium containing medications have caused magnesium toxicity and fatal hypermagnesemia, whereby magnesium serum concentrations have exceeded 2.6 mg/dl (Kutsal et al., 2007). Aside from the gastrointestinal disruptions, symptoms of hypermagnesemia may also include hypotension, difficulty breathing, lethargy, muscle weakness, irregular cardiac rhythms, and cardiac arrest (Musso, 2009).

4. Magnesium and Glycemic Control

Magnesium plays an important role in both glucose and insulin metabolism. It mediates glucose transporting mechanisms through cell membranes, through its effect on insulin signaling via tyrosine kinase activity, phosphorylase b kinase activity, and glucose transporter protein activity (Suarez et al., 1995; Barbagallo et al., 2003). Magnesium also acts as a cofactor for several enzymes involved in carbohydrate phosphorylation and oxidation, such as protein kinases and phosphatases, essential for glycolysis (Saris et al., 2000). Therefore, it enhances cellular uptake of glucose to optimize insulin sensitivity and action (Swaminathan, 2003).

Recent clinical studies have associated the role of magnesium deficiency and the resultant altered magnesium status as an independent risk factor for the development of metabolic syndrome and of each of its components (Larsson & Wolk, 2007; Song et al., 2005) whereby patients with metabolic syndrome were found to have low serum magnesium levels compared with healthy individuals (Haap et al., 2006). In brief, low magnesium status was found to be linked to the development of hypertension, insulin resistance, impaired glucose tolerance, dyslipidemia and central obesity (Belin & He, 2007). Magnesium supplementation of a meal increases postprandial magnesium levels and improves hyperlipidaemia in otherwise healthy subjects (Kishimoto et al., 2010). Similarly, magnesium was reported to reduce glucose tolerance to an OGTT (Zofková et al., 1987), and improve glucose disposal rate (Yajnik et al., 1984), while insulin levels were not altered and this may relate to an improvement in insulin sensitivity. Moreover, studies have revealed that hypomagnesemia was documented in patients diagnosed with glucose intolerance and type II diabetes (Pham et

al., 2007; Lima et al., 2009). In 2006, a meta-analysis of 9 randomized double-blind controlled studies of 370 patients revealed that oral magnesium supplementation for a period of 3 months significantly lowered fasting serum glucose levels in type II diabetic patients (Song et al., 2006).These protective and beneficial effects once again seem to be resulting from an improved insulin mediated glucose uptake, since magnesium has been shown to be vital for optimal coupling and signaling through insulin receptors (Song et al., 2006).

E. Potassium: The Mineral

The macro-mineral potassium is known to be the most abundant exchangeable cation in the human body (Thier, 1986). It is the main intracellular mineral existing at concentrations of 140 to 150 mEq/L, and only 3.5 to 5 mEq/L extracellularly (Thier, 1986). Serum potassium concentration is tightly regulated via homeostatic mechanisms within the narrow range of 3.5 to 5.5 mEq/L (Osorio & Linas, 1998). Hormones and chemicals such as the renin–angiotensin–aldosterone system, insulin, catecholamines and thyroid hormone affect the interchange between the intra and extra-cellular partitioning of potassium (Evans & Greenberg, 2005). Overall potassium status is influenced by several factors including dietary intake, urinary potassium excretion (kidneys are the chief regulators of potassium status), as well as other factors that affect potassium excretion (i.e. diuretics) (Chatterjee et al., 2011; Haas, 2011). Absorption of the mineral potassium from the intestine is virtually high, with around 90% absorption (Thier, 1986); however, it is one of the very soluble minerals, and can get easily lost in cooking and processing of foods (Haas, 2011).

1. Dietary Potassium

Adequate recommended intake of potassium for adults, according to the US Panel on Dietary Reference Intake is 4.7 grams per day, based on the health benefits of potassium related to hypertension, bone density and risk of kidney stones (IOM, 2004). Potassium can be found in a wide range of foods. Some of the richest sources of potassium are: green leafy vegetables such as parsley, spinach and lettuce, beans, broccoli, in addition to fruits such as, oranges, bananas, apples, and dried fruits (Haas, 2011). Whole grains are also a very rich source of potassium; as well as many meat foods such as salmon, beef, and cod, to name a few (Haas, 2011). It should be as well noted that caffeine and tobacco limit potassium absorption (Haas, 2011). Therefore, people at high risk for potassium insufficiency are alcoholics, heavy smokers, people on diuretics, and crash dieters.

2. Physiological Functions of Potassium

Potassium is crucial for proper conduction of nerve impulse, regulation of acid-base balance, and for maintenance of osmotic balance between cells and the interstitial fluid, via the action of Na+/K+-ATPase pump (Campbell, 1987). Potassium also plays a key role in membrane polarization, muscle contraction, and heartbeat regulation (Lockless et al., 2007). Metabolically, potassium contributes to protein synthesis and therefore is crucial for normal growth and muscle development (Haas, 2011). Moreover, potassium also participates in carbohydrate metabolism, particularly in the process of glycogenesis (Haas, 2011). Since the human body is highly dependent on potassium to maintain regular heart contraction and a healthy nervous system, it is necessary to ensure an adequate intake of this mineral.

Epidemiological and clinical studies have shown that sufficient intake of the mineral potassium has beneficial and protective effects on human health (He and MacGregor, 2008). One of the most pronounced effects is related to the inverse relation between potassium status and blood pressure among both hypertensive and non-hypertensives (He and MacGregor, 2008). Prospective cohort studies have likewise found a dose–response relationship between increasing potassium intake and risk of cardiovascular diseases (Dauchet et al., 2006). However, with all the technological advancements, cooking and processing of food have significantly decreased potassium content, along with the high consumption of processed foods and low consumption of fruits and vegetables. It has been revealed that at least in the USA and Canada, potassium dietary intake levels were much lower than the recommended levels (IOM, 2004).

3. Hypokalemia and Hyperkalemia

Potassium-related metabolic disorders can be categorized as those due to: dietary intake, altered excretion, or to deranged transcellular potassium shifts (Osorio & Linas, 1998). A decline in potassium levels whether due to vomiting, diarrhea, or diuresis might lead to a potentially fatal condition referred to as hypokalemia (Slonim et al., 2006). Symptoms may include cardiac arrhythmia and dysfunction, respiratory muscle paralysis, energy depletion and fatigue, loss of cellular integrity which can result in muscle necrosis, and paralytic ileus (Visveswaran, 2009). On the other hand, hyperkalemia is when the concentration of the electrolyte potassium in the blood is highly above the normal range. This condition can be caused by renal insufficiency, or excessive use of medication that intervene with urinary excretion such as ACE inhibitors and potassium-sparing diuretics, or by mineralocorticoid

(aldosterone) deficiency (Evans & Greenberg, 2005). Symptoms of hyperkalemia may include muscle weakness, palpitations, ventricular arthritis, cardiac arrhythmia, and even cardiac arrest (Evans & Greenberg, 2005).

4. Potassium and glycemic control:

One other effect is related to the involvement of potassium in glucose metabolism, in which potassium depletion resulting from a low potassium diet was reported to induce glucose intolerance that was associated with impaired insulin secretion, and was shown to be stimulated by potassium infusion (Rowe et al., 1980, Dluhy et al., 1972). In line with this, a strong relationship between thiazide-induced hypokalemia and glucose intolerance was reported in thiazide treated subjects (Zillich et al., 2006). Using thiazide diuretics as the first-line pharmacalogical treatment for hypertension has been granted authorization and support from national guidelines (Chobanian et al., 2003). However, data from recent clinical trials have revealed that thiazide-induced hypokalemia (with serum potassium concentration less than 3.5 mmol/L) is directly related to impaired glycemic control and eventually onset of type II diabetes (Verdecchia et al., 2005; Verdecchia et al., 2004). A recent analysis of 59 clinical trials revealed a strong association between hypokalaemia and glucose intolerance among those on thiazide diuretics (Zillich et al., 2006). Thiazde-induced hypokalemia results from an amplified renal excretion of potassium and the action of aldosterone in response to volume contraction, which further promotes potassium excretion (Zillich et al., 2006). In parallel to this, studies have shown that thiazde-induced hypokalemia and low serum potassium levels resulted in diminished insulin secretion levels from pancreatic beta cells, and hence increased

blood glucose levels (Rowe et al., 1980). Interestingly, experimentally induced hypokalemia have as well resulted in glucose intolerance due to a decline in pancreatic beta cells sensitivity to glucose loads, and thus low insulin secretion levels (Chatterjee et al., 2011). Moreover, following ingestion of potassium supplementation, defects in insulin secretion in response to hyperglycemia were corrected for, once again confirming that hypokalemia is what have caused prior glucose intolerance (Helderman et al., 1983). Current data therefore suggests the use of potassium supplementation or potassium-sparing diuretics while on thiazide diuretics, in order to improve glycemic control and perhaps prevent onset of diabetes (Zillich et al., 2006).

F. Incretins

Incretins are gut hormones that get post-prandially secreted from the gut into bloodstream, in response to enteral food ingestion, particularly to carbohydrates and fats. Studies have interestingly discovered that insulin secretion is provoked much more in response to oral nutrient ingestion compared to intravenous glucose infusion (Drucker, 2006). This insulin potentiating ability of incretins, known as the incretin effect, is responsible for 50% to 70% of the overall insulin secretion following oral glucose ingestion, beyond the secretion stimulated by the absorbed glucose itself (Vilsboll & Holst, 2004). Thereby incretins are considered to be insulinotropic hormones that tend to markedly stimulate insulin secretion (Kim & Egan, 2008).

The two main incretins are glucagon- like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). It has been speculated that an impaired incretin function may result in postprandial hyperglycemia and poor glycemic control; hence, it is plausible to deduce that incretins play a significant role in the pathophysiology of type 2 diabetes (Vilsboll & Holst, 2004). In light of these findings, two new classes of drugs have been introduced in 2005 that are based on incretin action, and are officially permitted to be consumed by type II diabetics to lower blood glucose levels: incretin enhancer 'sitagliptin', which is a DPP4 inhibitor – since GIP and GLP-1 are rapidly degraded by proteolytic enzyme dipeptidyl peptidase 4 (DPP4) and incretin mimetic 'exenatide' which is a potent long-acting agonist of the GLP-1 receptor (Kim & Egan, 2008).

*1. **Glucagon-like peptide-1 (GLP-1)***

GLP-1 is a 31-amino acid hormone secreted from the L cells of the distal ileum and colon (Baggio & Drucker, 2007). GLP-1 is produced from the cleavage of proglucagon. The glucagon gene, which encodes 158 amino acids, is not only expressed in the pancreatic alpha cells, but also in the L-cells of the gut. In the L cells of gut, proglucagon is cleaved to GLP-1, whereas in the pancreatic alpha cells, proglucagon cleavage yields glucagon (Vilsboll & Holst, 2004). The main physiological stimulus for the release of GLP-1 is food ingestion, especially if rich in carbohydrates or fats (Brubaker, 2006). The release of GLP-1 into bloodstream occurs in 2 phases, an early phase (10 to 15 minutes following meal ingestion) and a longer phase (30 to 60 minutes following meal ingestion) (Herrmann et al., 1995). GLP-1 is known to have a very short half life of 1.5 to 2 minutes, after which it gets cleaved by DPP-4, and thus becomes inactive (Kim & Egan, 2008). In humans, fasting plasma concentrations of bioactive GLP-1 range between 5 and 10 pmol/L and increase approximately 2 to 3 fold after a meal (Orskov et al., 1994), depending on the size and nutrient composition of the meal (Vilsboll et al., 2001). Upon its secretion, GLP-1 then stimulates insulin release from pancreatic beta cells, hinders glucagon release from pancreatic alpha cells, and decreases the rate of endogenous glucose production, all of which are channeled towards lowering blood glucose levels in type II diabetics (Prigeon et al., 2003). GLP-1 induces its insulinotropic effects by binding to its specific receptor (GLP-1R) (Kim & Egan, 2008). Apart from its direct insulinotropic role, GLP-1 slows gastric emptying, improves satiety, decreases food intake, and inhibits pancreatic cell apoptosis, thus as well improving glycemia and insulin resistance (Willms et al., 1996). Studies have demonstrated that intravenous infusion of GLP-1 slowed down gastric emptying, improved fasting glucose

levels, decreased postprandial glucose levels, and prevented the postprandial increase in triglycerides (Meier et al., 2006).

2. Glucose-Dependent Insulinotropic polypeptide (GIP)

GIP is a single 42-amino acid peptide synthesized from K cells located in the proximal small intestine, particularly the duodenum and jejunum (Kim & Egan, 2008). Similar to GLP-1, GIP is released into bloodstream in response to the oral ingestion of a carbohydrate or fat rich meal (Elliott et al., 1993), and induces its insulinotropic effect by binding to is its specific receptor (GIPR) (Kim & Egan, 2008). GIP not only stimulates pancreatic secretion of insulin, but it also accelerates the biosynthesis and transcription of proinsulin in the pancreatic ß cells, and prevents apoptosis of pancreatic beta cells, thereby improving overall insulin productivity (Baggio & Drucker, 2007). In 1996, a study revealed that administration of GIP antagonists suppressed insulin secretion by 72% (Tseng et al., 1999). Researchers have also found that GIP may account for up to 80% of the incretin mediated insulin secretion (Weaver et al., 2008). Apart from its insulinotropic responsibility, GIP also plays an important role in fat metabolism, whereby it stimulates insulin-mediated incorporation of fatty acids into triglycerides, activates lipoprotein lipase activity, and modulates fatty acid synthesis (Yip & Wolfe, 2000). GIP has a short half life that ranges between 5 to 7 minutes, after which it gets rapidly cleaved by DPP4, making it biologically inactive (Kim & Egan, 2008). GIP concentration in bloodstream is low in the fasting state, and rises within minutes of food ingestion, similar to GLP-1 (Drucker, 2006). Both GLP-1 and GIP induce insulin release via activation of their specific G protein-coupled receptors expressed directly on islet beta cells (Drucker, 2006).

CHAPTER III

MATERIALS AND METHODS

A. Study Design

This is a single blind, randomized cross over study, whereby each subject serves as his own control.

B. Subjects

Twelve healthy males recruited from advertisements, direct approach, and word of mouth were enrolled in this study. Subjects' age ranged between 21 to 29 years, and their BMI ranged between 22.0 and 29.0 kg/m^2. Exclusion criteria of this study included any significant medical or chronic diseases, regular use of medication that affects body weight, and weight loss of 3% or more in the preceding 3 months. Subjects were asked to maintain their regular dietary and physical activity habits during the entire study course, avoid alcohol consumption as well as any unusual strenuous exercise 24 hours prior to the study. Upon enrollment, all 12 subjects were asked to read all the terms and conditions for participation, and sign an informed consent (Appendix I and II). Study protocol was approved by the Institutional Review Board (IRB) committee at The American University of Beirut (AUB).

C. Study Protocol

The study took place at the Clinical Research Unit (CRU) at the American University of Beirut Medical Center (AUBMC). Participants were asked to complete a total of four visits, separated by a minimum of seven days. Subjects had to eat one of the 4 different types of bread on each visit: white bread, whole grain bread, restored bread, and fortified bread. The duration of each visit was around two hours. On each of the four visits, subjects had to come to the unit following 10 to 12 hours of an overnight fast. Upon arrival to the unit, anthropometric measurements (height, weight) were taken by the calibrated seca balance. An intravenous catheter was then inserted by a registered nurse for blood withdrawal via a 22 gauge needle inserted into the anticubital vein. A heplock was used to keep the needle locked for a two hour period. No heparin was added. Fasting blood (time 0) was withdrawn. Next, each subject (blinded to the type of bread he is eating) was given 90 grams of bread (45 grams of carbohydrates) to ingest within 10-15 min and subsequently drink 200ml of water. Blood samples were then collected at 15, 30, 45, 60, 90 and 120 minutes after meal ingestion. Water saline was infused between withdrawals to prevent vein obstruction. In total, blood was withdrawn at 7 different time intervals including the pre-meal blood withdrawal; a total of 10 ml was collected on each withdrawal. The collected blood samples were drawn into EDTA anticoagulant tubes and vacutainer tubes, and immediately inserted in a crushed ice container. Collected blood samples were then centrifuged for 15 minutes at 4^{o}C at 1000 RPM for serum separation. Serum was then stored in aliquots at -80 ^{o}C (until the end of the experiment). The serum was used to test for glucose, insulin, GLP-1, GIP, triglycerides, phosphorus, magnesium, and potassium.

D. Bread Making

Since the aim of this project was to fortify white bread with phosphorus, potassium and magnesium and test the resulting glycemic index of the bread, four types of bread (white bread, whole wheat bread, restored bread, and fortified bread) were made at the Food Processing Pilot Plant Department of the American University of Beirut. Locally produced white wheat flour (80% extraction) and whole grain wheat flour were purchased and stored at the department. White wheat flour (80% extraction) was fortified with minerals. Potassium phosphate was used as the source of phosphorus and potassium to fortify white flour. From previous experience, the addition of potassium phosphate to glucose solution was found to minimally affect the organoleptic properties; unlike that of sodium phosphate that was not well tolerated by subjects due to development of unfavorable flavor (fishy flavor). Magnesium carbonate was used as the source of magnesium. Magnesium carbonate is known to be highly soluble and is commonly used as a supplement and fortificant.

1. ***White Bread (WB)***

Ingredients of white bread included: 3 kg white flour, 60 g sugar (20g per kg of flour, 48 g of salt, 30 g yeast, and 1680 ml of water was added to the mixture. Ingredients were then mixed in a dough mixer (DITO SAMA, Model BM 20S, France) at low speed for 7 min until a smooth continuous dough was obtained. Dough was then put into an incubator and fermented at 40 ^{o}C for 15 minutes. Next, dough was divided into equal balls and the dough balls were proofed again at 40 ^{o}C for 30 minutes. The balls were flattened into thin sheets, put

back once again into the incubator at 40 ^{o}C for 15 minutes and baked at 500 ^{o}C until optimum crust color was reached.

2. ***Whole Grain Bread (WG)***

Ingredients of whole grain bread included: 3 kg whole grain flour, 60 g sugar (20g per kg of flour, 48 g of salt, 30 g yeast, and 1850 ml of water was added to the mixture. The procedure for the making of the bread is the same as that of the control White Bread (WB).

3. ***White Bread Restoration (WB-R)***

White bread restoration was achieved by fortifying white wheat flour (80% extraction) with minerals to a level that restores its content of minerals to that of whole grain wheat flour. Based on data from Table 4, white flour restoration requires the addition of 250 mg of P/100g white flour (total P =358mg/100g) and 100 mg of Mg/100g white flour (total Mg =122mg/100g).

Ingredients of restored white bread included: 3 kg white flour, 60 g sugar (20g per kg of flour, 48 g of salt, 30 g yeast, 11 g of magnesium carbonate, 33 g of potassium phosphate, and 1650 ml of water was added to the mixture. The procedure for the making of the bread is the same as that of the control White Bread (WB).

4. ***White Bread Fortification (WB-F)***

White bread fortification is achieved by fortifying white wheat flour (80% extraction) with minerals to a level that is 100% higher than that of whole grain wheat flour. Based on data from Table 4, white flour fortification requires the addition of 580 mg of

P/100g white flour (total P = 688mg/100g) and 250 mg of Mg/100g white flour (total Mg = 272mg/100g). It is worth noting that these doses are still considered to be safe and do not exceed the tolerable upper limits of both minerals (UL of P = 4 grams/day, UL of Mg from supplements only = 350 mg/day).

Ingredients of fortified white bread included: 3 kg white flour, 60 g sugar (20g per kg of flour, 48 g of salt, 30 g yeast, 26 g of magnesium carbonate, 76 g of potassium phosphate, and 1700 ml of water was added to the mixture. The procedure for the making of the bread is the same as that of the control White Bread (WB).

E. Analytical Procedures

1. Glucose, triglycerides, total phosphate, potassium, and magnesium

Glucose, triglycerides, total phosphate, potassium and magnesium were measured via the Virtos machine 350 by Ortho-Clinical Diagnostics, Johnson & Johnson, New York.

2. Insulin, GLP-1, and GIP

Serum insulin, GLP-1, and GIP levels were measured via the ELISA kit from Diametra Millipore Corporation, Billerica, USA.

3. Area Under the Curve and Glycemic Index

Area under the curve was calculated according to the trapezoidal rule in geometry (Brouns et al., 2005). The GI rating (%) of a test food (bread type) was then calculated by dividing the AUC for the test food by the AUC for the reference food (white bread), and

multiplying it by 100. The average GI value was then determined from data collected from all subjects (n=12).

4. Statistical analysis

Minitab 16 was used to analyze all data. Paired t-test was used to compare differences between bread groups (WG), (WB-R), (WB-F) and control bread group (WB), at each time point. One-way ANOVA via Fisher's method was then used to detect statistical significance within the same bread group, at different time intervals. The General Linear Model (GLM) via the two-way analysis of variance was used as well to determine statistical significance with effects for Bread group, Time, and Bread group by Time interaction. For all data, statistical significance is at the level of 5% (p-value <0.05).

CHAPTER IV

RESULTS

A. Subjects characteristics:

A total of twelve male subjects were enrolled in this study. All subjects (n=12) matched the inclusion criteria previously assigned for this study. Participants did not encounter any particular discomfort during blood withdrawal in all four visits. Baseline and fasting characteristics of all subjects are listed in Table 5. Mean fasting blood parameters of all subjects were all within normal ranges. Subjects' BMI ranged between normal to overweight; however, overall mean indicates an overweight BMI.

Table 5: Subjects' characteristics:

n=12	**Mean**	**SEM**	**Minimum**	**Maximum**
Age (yrs)	24.5	1.02	21	33
Weight (kg)	83.92	3.35	65.7	104.7
Height (m)	1.79	0.017	1.64	1.89
BMI (kg/m2)	26.03	0.672	22.02	29.63
Fasting glucose (mg/dl)	94.00	3.28	74.75	112
Fasting insulin (μIU/mL)	6.679	1.92	1.37	24.12
Fasting triglycerides (mg/dl)	91.47	3.78	47.3	157
Fasting serum phosphate (mg/dl)	3.79	0.191	2.9	5.2

SEM = standard error of the mean

B. Postprandial blood parameters response

1. Postprandial inorganic phosphate response:

Mean serum phosphate levels failed to show any statistical difference at different time intervals within the same bread group (Table 6). Throughout all time intervals, mean serum phosphate levels were higher in WB-R and WB-F as compared to WB and WG (Figure 3). After consumption of both WB and WG, mean serum phosphate levels continued to decrease gradually across all time intervals to reach levels of 3.27 mg/dl and 3.2 mg/dl, respectively. The decrease in serum phosphate levels in the WB-R and WB-F groups however, was followed by an increase at 45 and 90 minutes; to reach levels of 3.63 mg/dl and 3.84 mg/dl respectively (Table 6). When comparing bread groups to control test food (WB), both WB-R and WB-F showed significantly higher levels of serum phosphate at time 120. The phosphate change curve showed statistical difference at different time intervals within the WG group (Table 7, Figure 4). Serum phosphate change also showed that serum phosphate level significantly decreased in the WG group at time 60 and 90 when compared to WB, and in the WB-F group at time 120 when compared to WB. Correspondingly, phosphate change curves also showed statistical significance within bread groups and across time intervals when analyzed by GLM (two-way analysis of variance).

2. Postprandial serum magnesium response:

No statistical significance was detected between different time intervals within any of the bread groups neither in mean serum magnesium levels nor change (tables 8 and 9). However, when analyzed by two-way analysis of variance, magnesium change curves

showed significance within bread groups and across time intervals. The WG group plotted the lowest serum magnesium levels (Figure 5), with a statistical significance being detected at time 90 as compared to WB. Mean magnesium levels increased gradually following ingestion of WB to reach a level of 2.13 mg/dl, and then faced a decline at time 90. Following ingestion of WB-F, mean magnesium serum levels experienced a significant decrease (as revealed by mean magnesium change) followed by an uninterrupted pick up at time 45 to further reach 2.13 mg/dl (Figure 5). Serum magnesium change significantly decreased in the WB-F group at time 45 when compared to WB (Figure 6).

3. Postprandial serum potassium response:

Baseline and postprandial mean serum potassium levels are presented in Table 10. Significant statistical difference was shown at different time intervals within the WG group. Statistical significance was as well determined across the three bread groups when compared to WB. At baseline, all three bread groups had a significantly higher potassium mean than WB. As shown in Figure 7, WB-F maintained a level of postprandial potassium that is significantly higher than that of WB at time 45, 60, 90, and 120. After their ingestion, WB and WG groups experienced a continuous gradual decrease in potassium levels. Mean potassium change also showed significant statistical difference at different time intervals within the WG group (Table 11). Serum potassium change significantly decreased in the WG group at time 60 and 90 when compared to WB (Figure 8).

Table 6: Mean serum phosphate levels following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	3.68±0.16	3.43±0.18	3.43±0.24	3.44±0.22	3.4±0.19	3.41±0.16	3.27±0.16	0.859
WG	3.79±0.23	3.41±0.21	3.41±0.22	3.29±0.18	3.16±0.19[a]	3.15±0.19	3.2±0.19	0.302
WB-R	3.95±0.20	3.62±0.17	3.54±0.18	3.75±0.19	3.55±0.16	3.53±0.17	3.63±0.19[a]	0.67
WB-F	3.84±0.19	3.71±0.18	3.72±0.24	3.61±0.19	3.67±0.22	3.75±0.26	3.84±0.27[b]	0.99

Values are mean serum phosphate (mg/dl) ± standard error of the mean.

[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

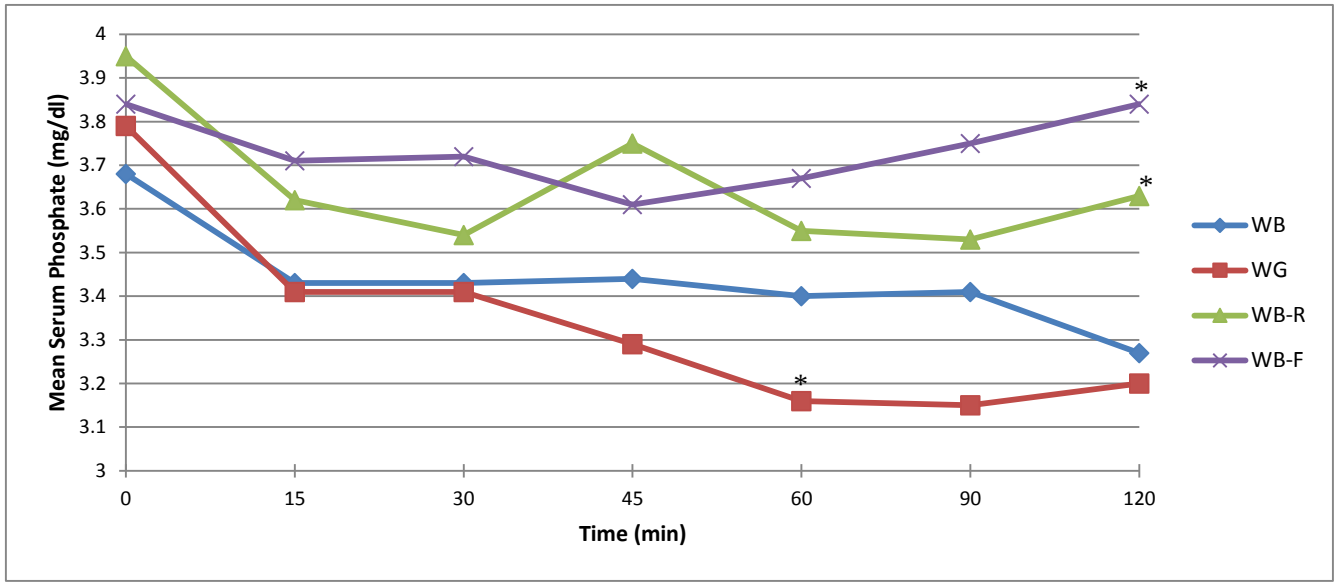


Figure 3: Mean serum phosphate curves

* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 7: Mean serum phosphate change following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	0.00±0.00	-0.25±0.09	-0.25±0.15	-0.24±0.13	-0.28±0.09	-0.28±0.1	-0.41±0.1	0.225
WG	0.00±0.00	-0.38±0.04	-0.38±0.11	-0.5±0.07	-0.63±0.07[a]	-0.64±0.07[a]	-0.59±0.08	<0.001*
WB-R	0.00±0.00	-0.33±0.13	-0.41±0.13	-0.2±0.17	-0.4±0.16	-0.41±0.16	-0.32±0.19	0.387
WB-F	0.00±0.00	-0.13±0.08	-0.13±0.12	-0.23±0.07	-0.16±0.09	-0.09±0.16	0.00±0.179[a]	0.757

Values are mean serum phosphate change ± standard error of the mean.

[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

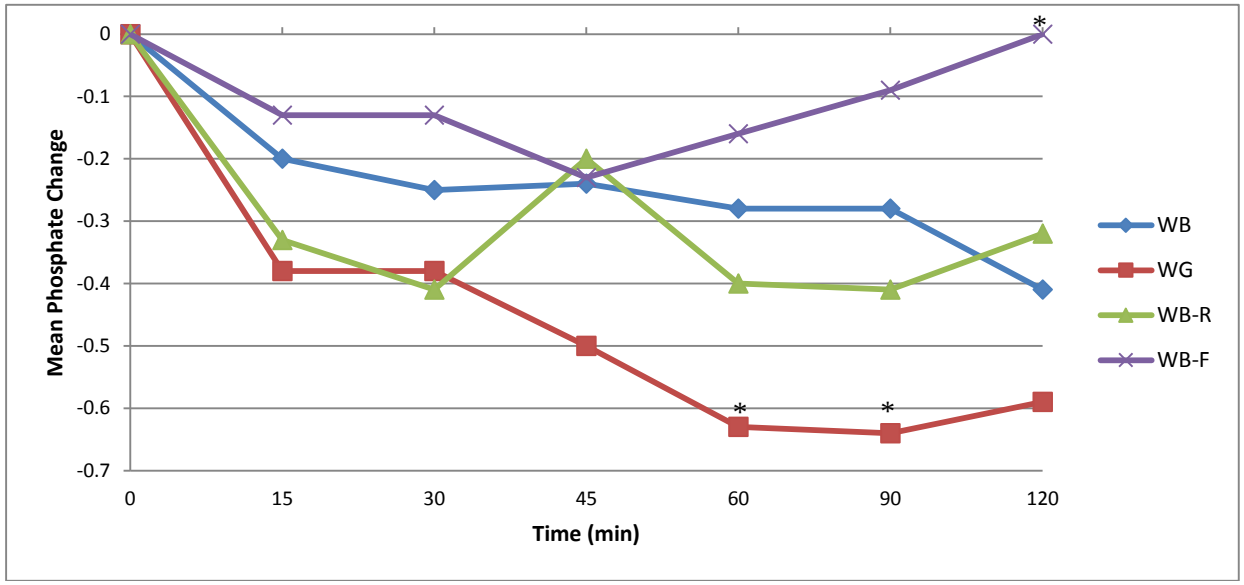


Figure 4: Mean serum phosphate change curves

* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 8: Mean serum magnesium levels following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	1.95±0.06	1.96±0.07	1.98±0.08	2.05±0.08	2.06±0.07	2.13±0.07	2.03±0.06	0.581
WG	1.94±0.06	1.91±0.06	1.94±0.08	1.91±0.04	1.93±0.05	1.96±0.05[a]	1.98±0.05	0.980
WB-R	2.0±0.05	1.99±0.07	1.94±0.05	2.12±0.08	2.05±0.05	2.11±0.04	2.13±0.06	0.214
WB-F	2.04±0.06	2.05±0.1	2.03±0.1	1.98±0.06	2.06±0.08	2.13±0.11	2.13±0.11	0.910

Values are mean serum magnesium (mg/dl) ± standard error of the mean.
[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

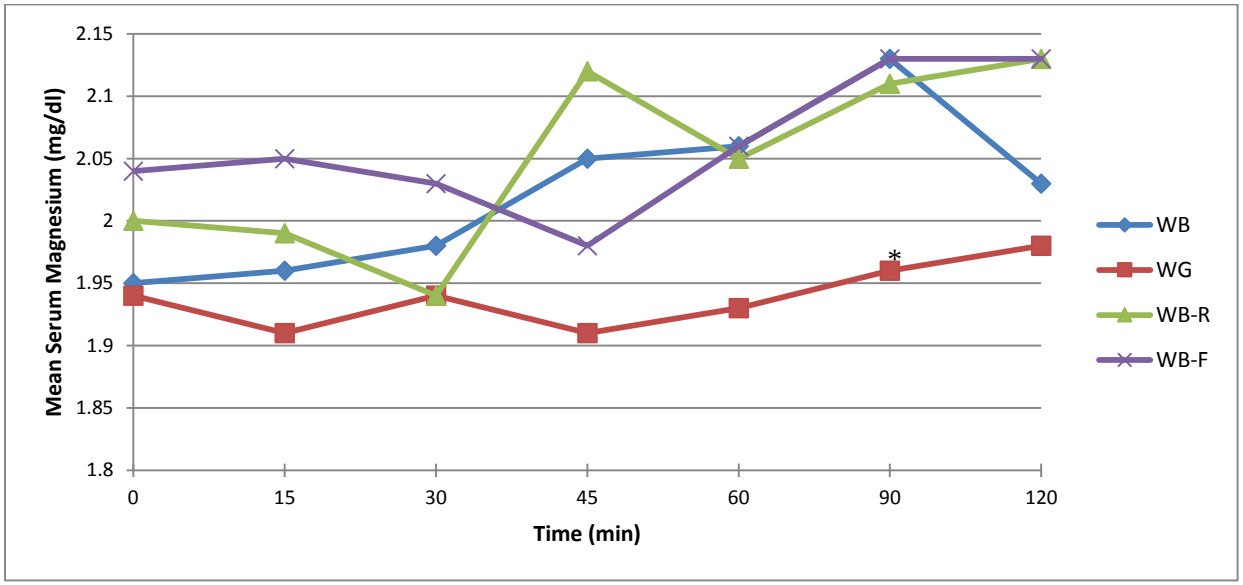


Figure 5: Mean serum magnesium curves
* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 9: Mean serum magnesium change following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	0.00±0.00	0.02±0.04	0.03±0.07	0.1±0.07	0.11±00.06	0.18±0.07	0.08±0.04	0.278
WG	0.00±0.00	-0.03±0.03	0.00±0.06	-0.03±0.04	-0.02±0.03	0.02±0.04	0.03±0.04	0.869
WB-R	0.00±0.00	-0.01±0.04	-0.06±0.03	0.11±0.09	0.05±0.06	0.12±0.08	0.13±0.08	0.254
WB-F	0.00±0.00	0.01±0.05	-0.01±0.06	-0.07±0.03[a]	0.02±0.03	0.08±0.06	0.09±0.07	0.283

Values are mean serum magnesium change ± standard error of the mean.
[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

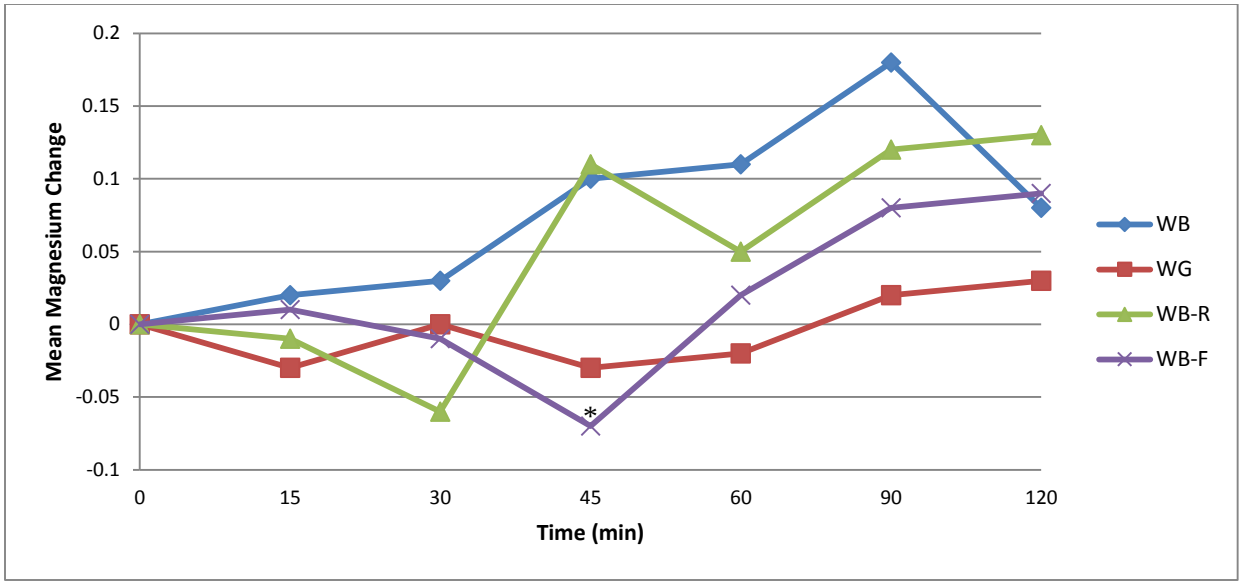


Figure 6: Mean serum magnesium change curves
* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 10: Mean serum potassium levels following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	4.75±0.11	5.01±0.22	4.73±0.21	4.59±0.2	4.54±0.18	4.42±0.15	4.37±0.15	0.183
WG	5.18±0.18[b]	5.08±0.19	4.85±0.19	4.76±0.17	4.58±0.14	4.49±0.11	4.51±0.14	0.013*
WB-R	5.3±0.22[a]	4.68±0.41	4.87±0.17	4.8±0.22	4.73±0.19	4.75±0.26	4.78±0.31	0.714
WB-F	5.28±0.17[a]	5.07±0.28	5.07±0.23	5.08±0.22[b]	4.93±0.17[a]	4.91±0.19[b]	4.93±0.25[b]	0.908

Values are mean serum potassium (mmol/L) ± standard error of the mean.
[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

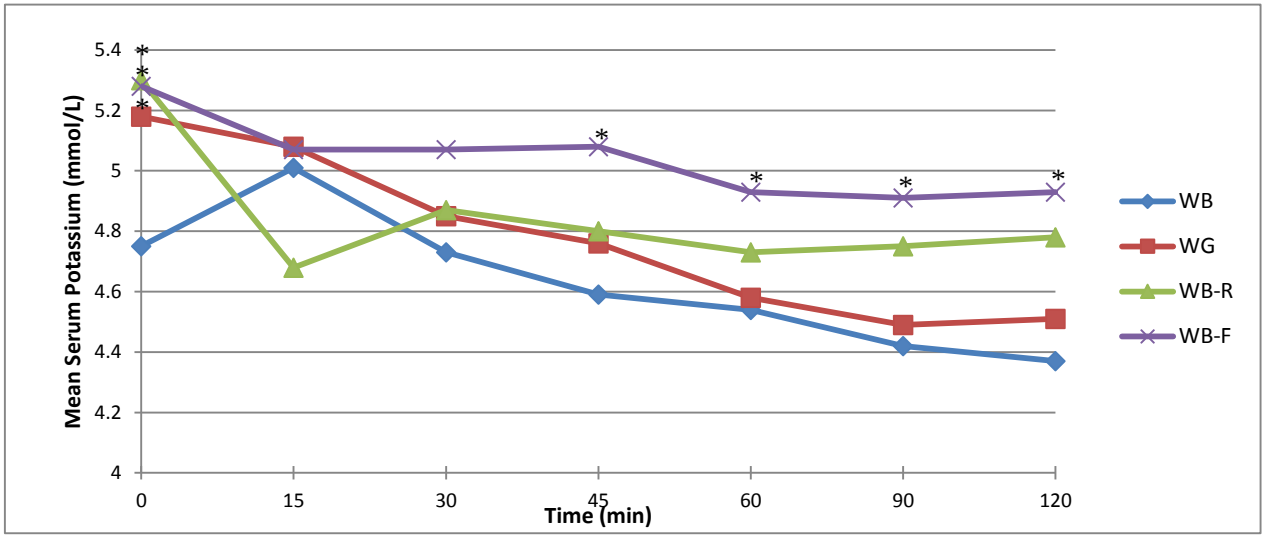


Figure 7: Mean serum potassium curves
* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 11: Mean serum potassium change following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	0.00±0.00	0.26±0.2	-0.02±0.19	-0.16±0.16	-0.21±0.17	-0.33±0.18	-0.38±0.14	0.068
WG	0.00±0.00	-0.09±0.19	-0.33±0.2	-0.42±0.19	-0.6±0.18[a]	-0.72±0.14[a]	-0.67±0.13	0.010*
WB-R	0.00±0.00	-0.62±0.23[b]	-0.43±0.15	-0.5±0.18	-0.57±0.2	-0.55±0.20	-0.53±0.25	0.294
WB-F	0.00±0.00	-0.21±0.2	-0.26±0.19	-0.19±0.21	-0.35±0.22	-0.37±0.23	-0.35±0.26	0.87

Values are mean serum potassium change ± standard error of the mean.
[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

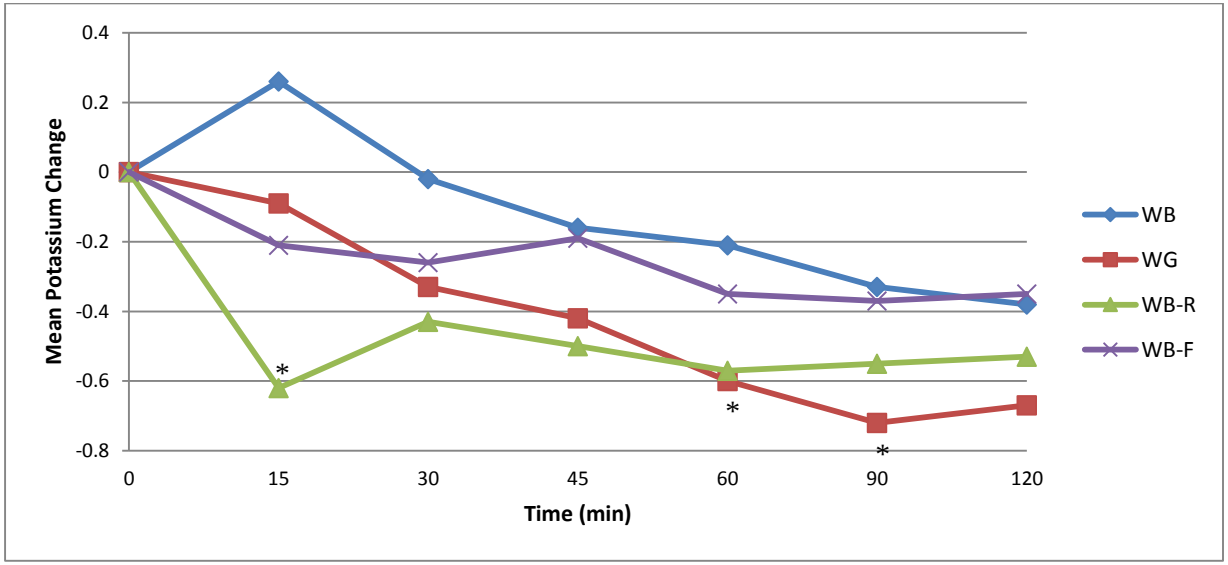


Figure 8: Mean serum potassium change curves
* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

4. Postprandial glucose response:

Postprandial glucose levels following ingestion of all bread groups are presented in Table 12 and Figure 9. Mean serum glucose levels were significantly different at different time intervals within each of the bread groups. Correspondingly, when analyzed by two way analysis of variance, mean serum glucose levels and change revealed statistical significance among bread group by time interaction. Glucose levels have peaked at 30 minutes post ingestion, and began to decrease at 60 minutes. At 15 minutes, WB-F has significantly reached the highest serum glucose levels and change (Table 12). At 30 minutes, WB-R significantly attained the highest glucose levels. In both mean serum glucose absolute levels and change, statistical significance was determined between control test food (WB) and WG at 120 minutes; between control test food and WB-R at 30, 90, and 120 minutes; between control test food and WB-F at 15, 60, 90, and 120 minutes. At 60 minutes and on, WB-R and WB-F significantly retained the lowest mean serum glucose levels and lowest change (Figures 9 and 10). It is worth noting that throughout all time intervals, WG and WB-R followed an analogous trend.

5. Postprandial insulin response:

Mean serum insulin levels are presented in Table 14 and Figure 11. Similar to glucose, insulin levels peaked at 30 minutes, except for the WG which peaked at 60 minutes. Results did not show any statistical significance across the four bread groups; however insulin levels were significantly different at different time intervals –within the same bread group in both mean serum insulin levels and mean serum insulin change (Tables 14 and 15). No significance was determined when comparing bread groups to control test food (WB).

6. Postprandial serum triglyceride response:

Neither mean serum triglycerides levels nor mean serum triglycerides change showed any statistical difference across the four bread groups when analyzed by paired t-test (Tables 16 and 17). Likewise, no significant changes were detected at the different time intervals within the same bread group when analyzed using one way ANOVA. However, mean serum triglycerides change showed strong statistical significance with effect to bread groups, and time, when analyzed by two-way analysis of variance; thereby suggesting that WB-R and WF-R significantly retained the lowest levels of triglycerides.

Table 12: Mean serum glucose levels following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	95.00±3.11	100.17±3.58	114.17±3.42	118.33±4.03	117.75±5.15	110.67±4.49	108.92±3.9	<0.001*
WG	93.75±4.62	101.92±4.39	121.33±4.43	121.83±5.66	111.83±5.45	105.42±4.26	97.5±4.83[a]	<0.001*
WB-R	93.25±2.30	98.25±2.27	122.75±3.47[a]	122.00±5.15	110.42±4.64	100.58±2.86[a]	96.25±1.61[b]	<0.001*
WB-F	94.00±3.10	107.58±3.65[a]	121.25±4.12	120.67±5.45	108.08±4.31[a]	96.58±4.00b	82.25±3.87[c]	<0.001*

Values are mean serum glucose (mg/dl) ± standard error of the mean.

[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

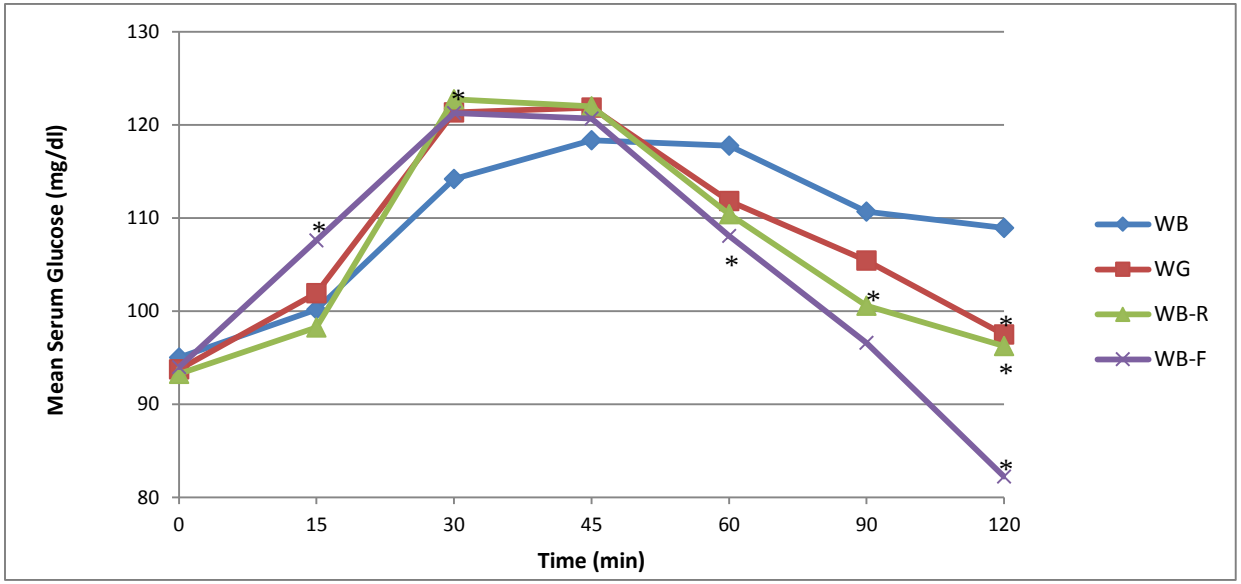


Figure 9: Mean serum glucose curves

* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test

Table 13: Mean serum glucose change following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	0.00±0.00	5.17±3.63	19.17±3.24	23.33±3.16	22.75±3.74	15.67±3.02	13.92±1.73	<0.001*
WG	0.00±0.00	8.17±2.75	27.58±2.98	28.08±3.84	18.08±2.29	11.67±3.45	3.75±1.01[c]	<0.001*
WB-R	0.00±0.00	5.00±2.94	29.50±3.88[a]	28.75±5.46	17.17±5.29	7.33±3.72[a]	3.00±1.91[c]	<0.001*
WB-F	0.00±0.00	13.58±3.00[a]	27.25±2.75	26.67±3.25	14.08±3.70[a]	2.58±3.29[b]	-11.75±2.05[c]	<0.001*

Values are mean serum glucose change ± standard error of the mean.

[a, b, c] Values are significantly different from control test food (WB) within a specific time at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

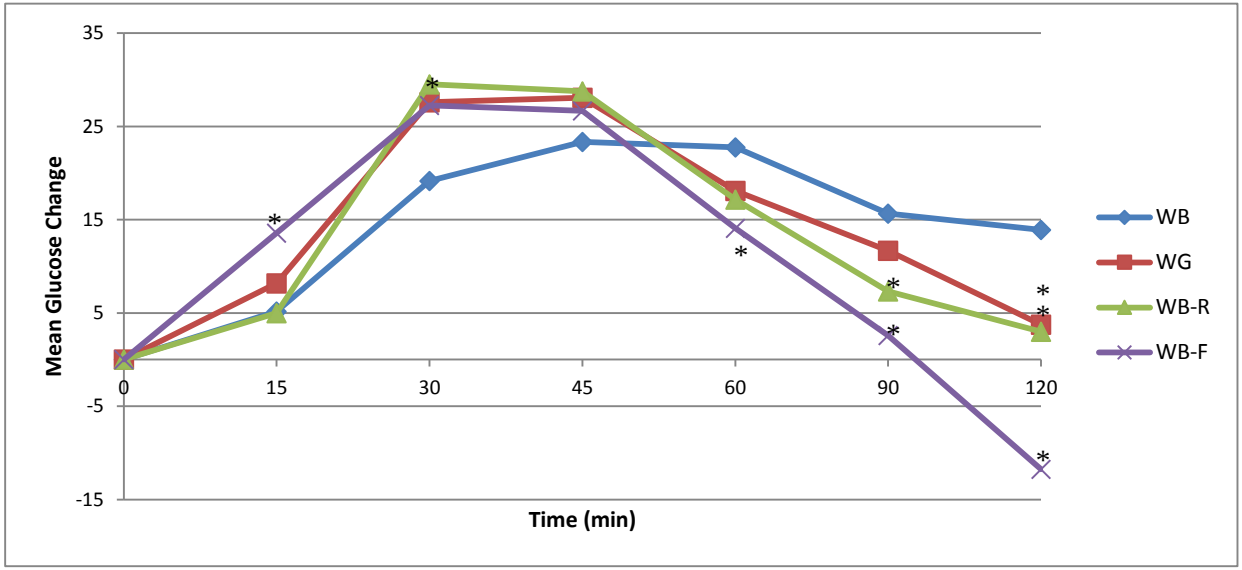


Figure 10: Mean serum glucose change curves

* Values are significantly different from control food (WB) at a specific time, at $p<0.05$ using paired t-test.

Table 14: Mean serum insulin levels following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	4.84±0.71	33.13±5.81	29.98±4.93	<0.001*
WG	6.53±1.07	37.3±4.71	45.48±8.08	<0.001*
WB-R	6.49±2.17	37.99±7.52	31.88±6.34	0.001*
WB-F	8.85±3.73	35.06±6.34	35.95±5.82	0.001*

Values are mean serum insulin (µIU⁄ml) ± standard error of the mean.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

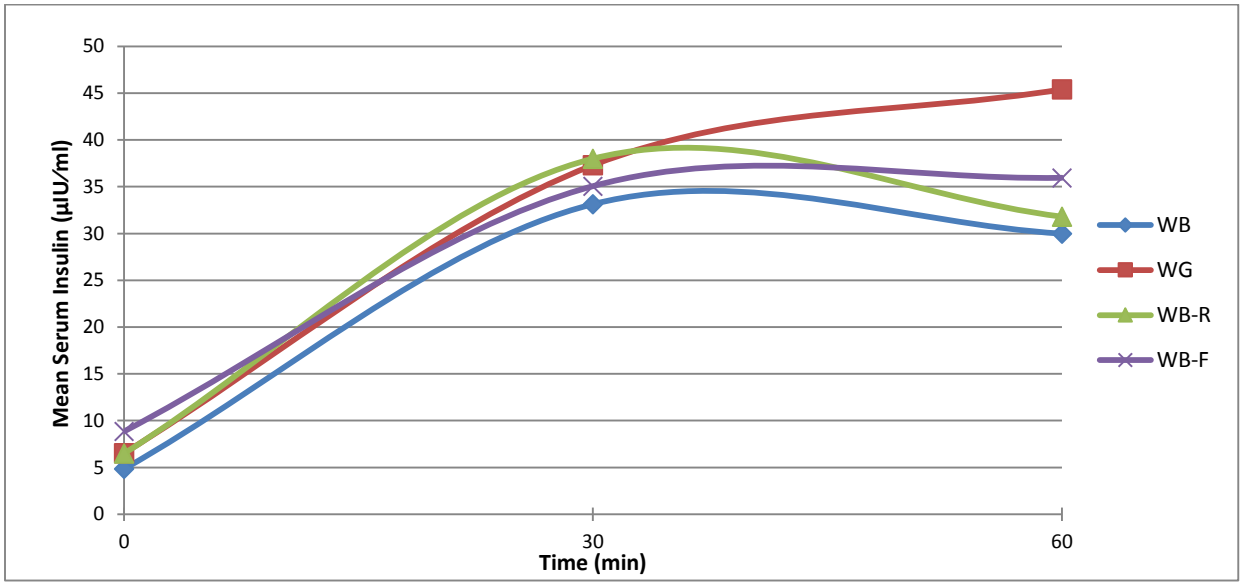


Figure 11: Mean serum insulin curves

Table 15: Mean serum insulin change following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	0.00±0.00	28.29±5.29	25.14±4.39	<0.001*
WG	0.00±0.00	30.77±4.13	39.05±7.6	<0.001*
WB-R	0.00±0.00	31.6±6.79	25.39±6.08	<0.001*
WB-F	0.00±0.00	26.2±5.06	27.1±4.58	<0.001*

Values are mean serum insulin change ± standard error of the mean.

* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

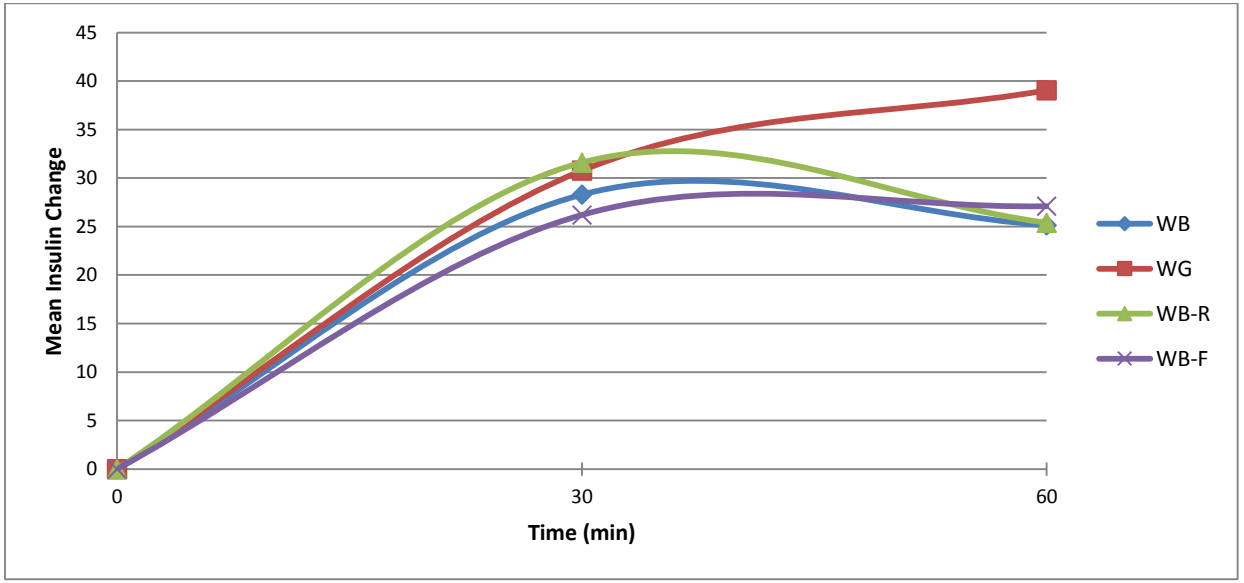


Figure 12: Mean serum insulin change curves

Table 16: Mean serum triglycerides levels following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	94.42±8.33	88.0±8.71	89.92±7.81	96.9±10.3	97.3±11.4	93.23±9.73	84.75±8.64	0.957
WG	78.67±7.65	73.33±6.53	79.42±7.21	79.67±7.38	80.08±7.84	77.50±7.28	74.5±7.11	0.992
WB-R	101.0±13.2	90.0±11.3	85.3±10.2	94.9±10.6	90.2±12.2	85.4±11.4	85.4±11.2	0.955
WB-F	91.8±10.9	90.9±14.2	86.0±12.7	82.1±10.8	84.3±12.0	82.4±12.8	81.8±13.4	0.995

Values are mean serum triglycerides ± standard error of the mean

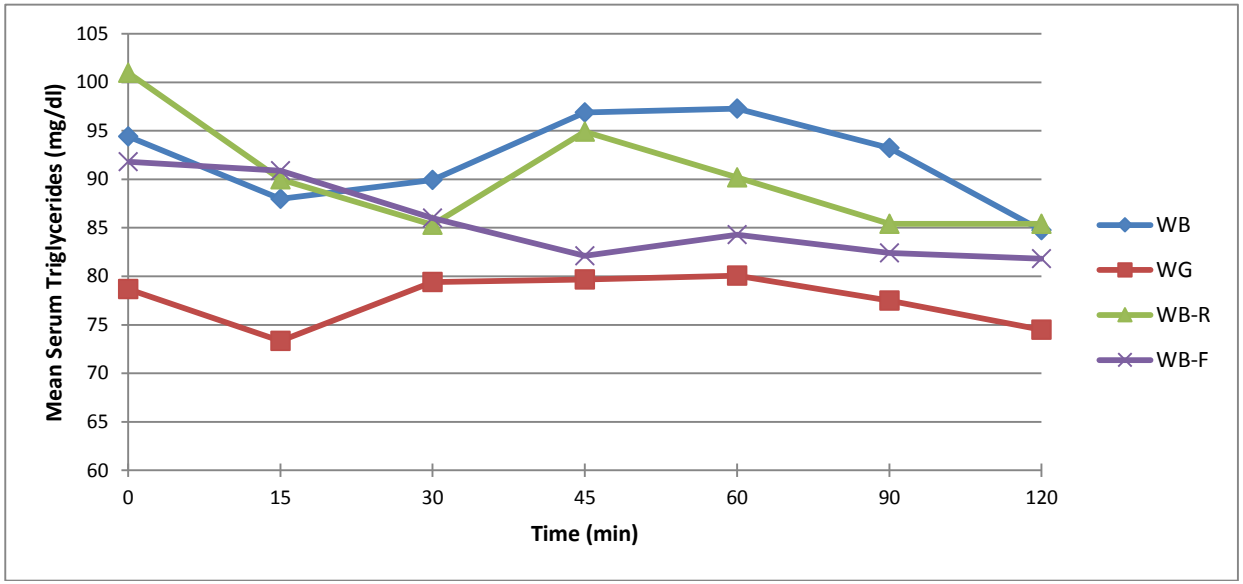


Figure 13: Mean serum triglycerides curves

Table 17: Mean serum triglycerides change following the ingestion of each of the bread types:

Bread Group	0 min	15 min	30 min	45 min	60 min	90 min	120 min	p-value
WB	0.00±0.00	-6.42±3.45	-4.5±3.82	2.5±6.19	2.83±5.66	-1.17±3.54	-9.67±3.34	0.331
WG	0.00±0.00	-5.33±2.39	0.75±3.27	1.00±2.27	1.42±3.05	-1.17±3.54	-4.17±4.2	0.561
WB-R	0.00±0.00	-11.00±2.8	-15.75±3.86	-6.58±6.77	-10.83±6.31	-15.58±5.87	-15.58±8.02	0.34
WB-F	0.00±0.00	-0.92±5.34	-5.83±2.84	-9.75±3.58	-7.58±2.57	-9.42±3.46	-10.00±4.08	0.204

Values are mean serum triglycerides change ± standard error of the mean

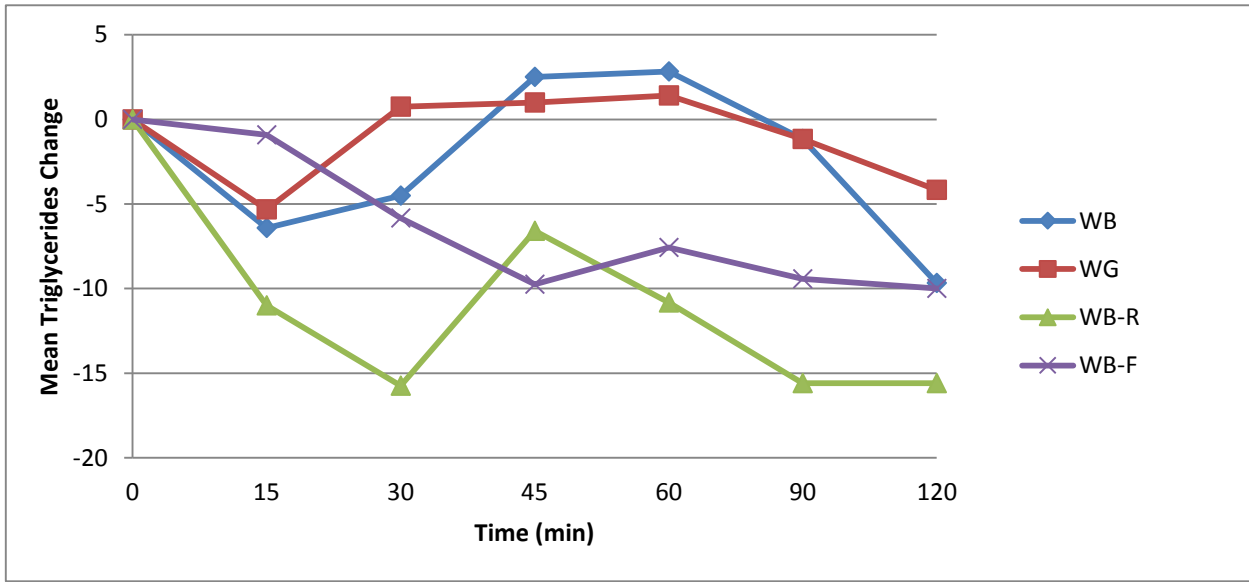


Figure 14: Mean serum triglycerides change curves

7. Postprandial GLP-1 and GIP responses:

Mean serum GLP-1 levels showed statistical significance at different time intervals within the WG group and the WB-R group independently (Table 18). No statistical significance was detected when comparing the four bread groups to each other. Mean GLP-1 levels peaked at 30 minutes in all four groups, with WB-R having reached the highest level, yet without attaining any statistical significance (Figure 15). Mean serum GLP-1 change showed statistical significance at different time intervals within the WG, WB-R, and WB-F groups independently (Table 19). Similar to GLP-1, GIP showed statistical significance at different time intervals within each of WG, WB-R, and WB-F groups (Table 20, Figure 17). No statistical significance was detected when comparing the four bread groups to each other. Mean GIP levels kept increasing till time 60 in all four groups. Mean serum GIP change showed statistical significance at different time intervals within the WB-R and WB-F groups independently (Table 21).

Table 18: Mean serum GLP-1 levels following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	29.96±3.29	37.37±4.64	26.23±2.91	0.088
WG	28.72±2.96	37.95±4.09	26.3±2.47	0.039*
WB-R	30.71±3.01	44.08±3.05	31.18±3.74	0.014*
WB-F	27.62±3.34	35.82±3.74	27.56±2.68	0.139

Values are mean serum GLP-1 (pM) ± standard error of the mean.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

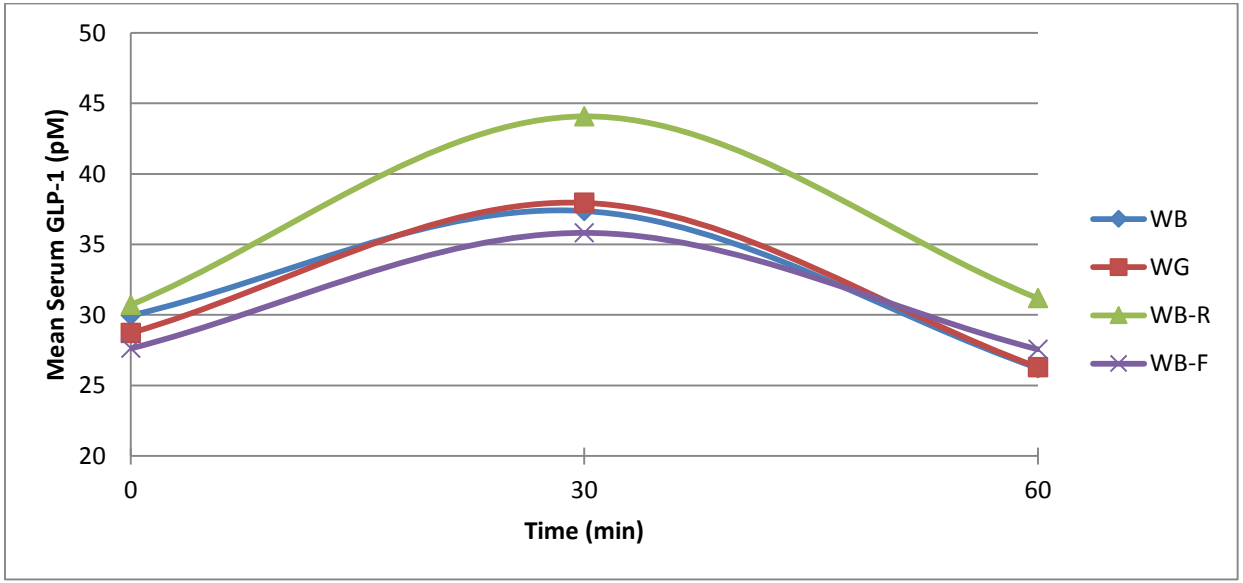


Figure 15: Mean serum GLP-1 curves

Table 19: Mean serum GLP-1 change following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	0.00±0.00	7.41±2.99	-3.73±1.42	0.074
WG	0.00±0.00	9.23±3.82	-2.42±2.02	0.006*
WB-R	0.00±0.00	13.37±2.49	0.46±3.82	0.001*
WB-F	0.00±0.00	8.21±1.9	-0.06±2.54	0.003*

Values are mean serum GLP-1 change ± standard error of the mean.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

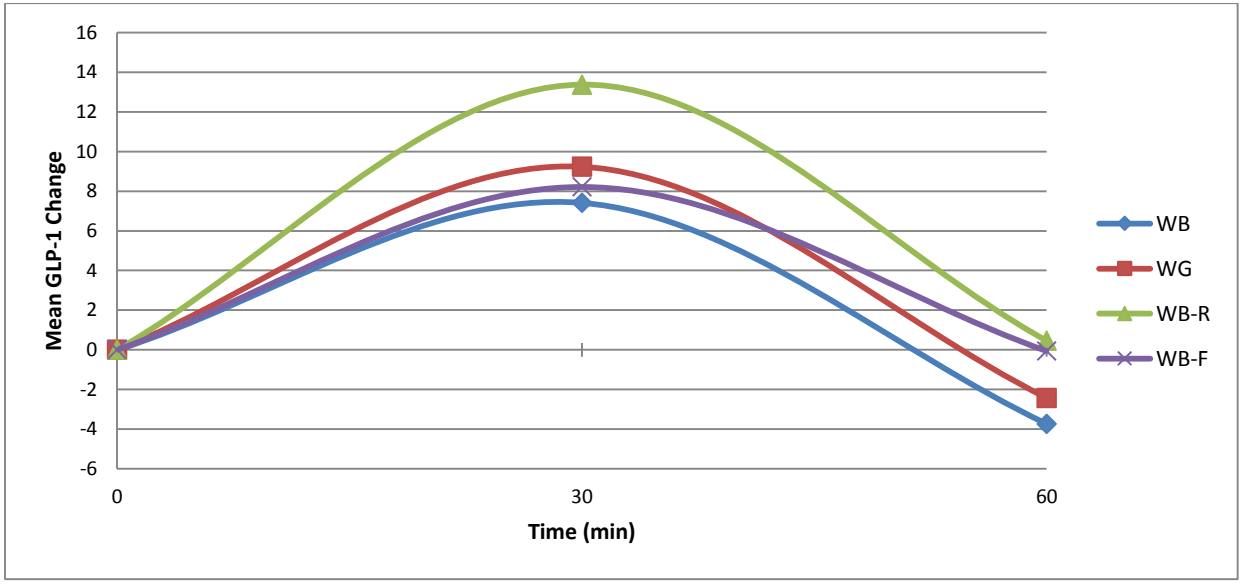


Figure 16: Mean serum GLP-1 change curves

Table 20: Mean serum GIP levels following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	85.4±22.4	271.7±50.9	270.4±32.6	0.203
WG	145.8±57.2	261.4±28.7	313.5±29.1	0.019*
WB-R	83.9±18.2	265.3±37.2	272.6±36.5	<0.001*
WB-F	66.6±15.3	238.8±38.7	241.5±33.6	<0.001*

Values are mean serum GIP (pg/ml) ± standard error of the mean.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

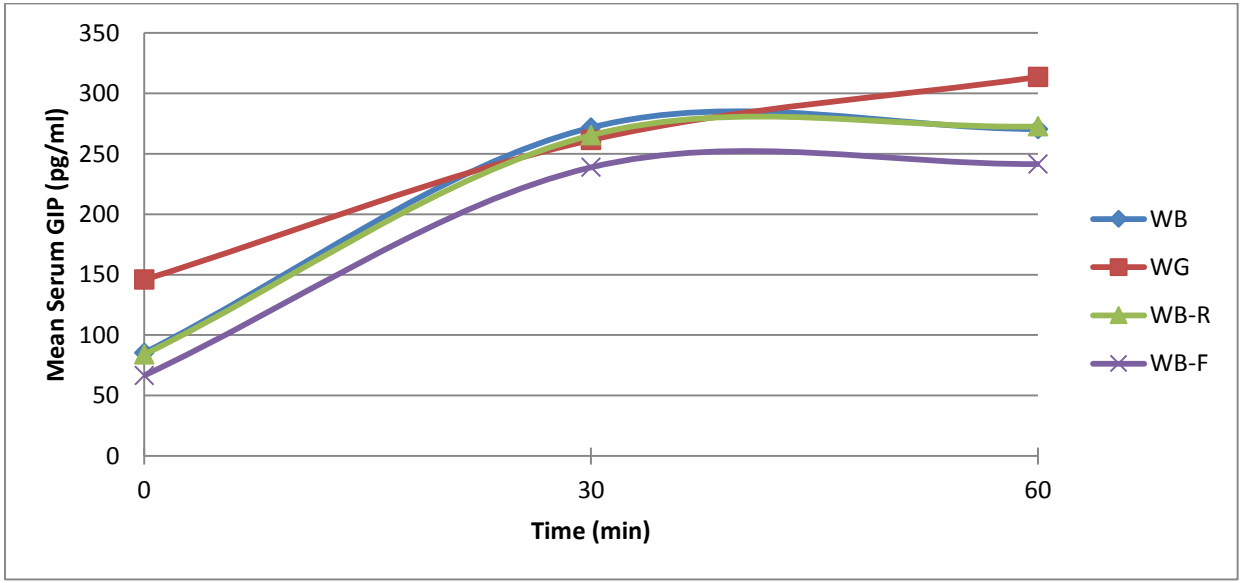


Figure 17: Mean serum GIP curves

Table 21: Mean serum GIP change following the ingestion of each of the bread types:

Bread Group	0 min	30 min	60 min	p-value
WB	0.00±0.00	186.3±37.1	185.0±15.7	0.076
WG	0.00±0.00	115.6±64.3	167.7±70.9	0.105
WB-R	0.00±0.00	181.5±27.3	188.7±24.4	<0.001*
WB-F	0.00±0.00	172.1±26.1	174.8±24.7	<0.001*

Values are mean serum GIP change ± standard error of the mean.
* Statistical significance at the level of 5% (p-value <0.05) between the different time intervals within the same bread group using one-way ANOVA.

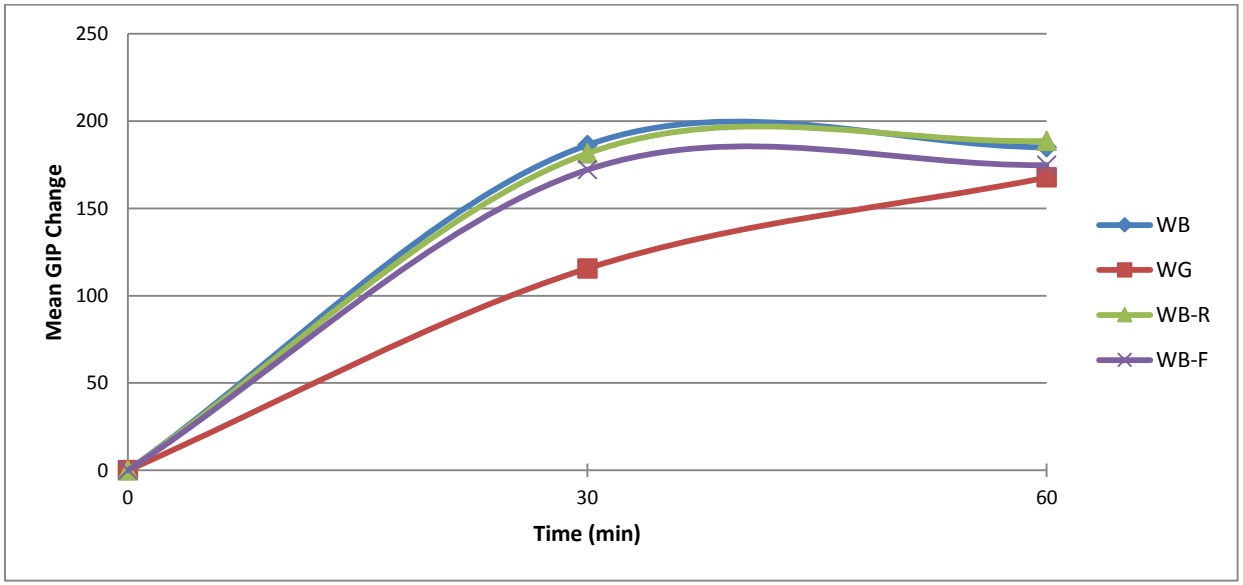


Figure 18: Mean serum GIP change curves

Table 22: Two-way analyses of variance:

	Bread Group	Time	Group-Time
Glucose	0.144	0.000*	0.033*
Δ Glucose	0.073	0.000*	0.000*
Insulin	0.091	0.002*	0.763
Δ Insulin	0.121	0.034*	0.541
Triglycerides	0.044*	0.896	1.000
Δ Triglycerides	0.000*	0.034*	0.712
Phosphate	0.001*	0.155	0.999
Δ Phosphate	0.000*	0.000*	0.624
Potassium	0.005*	0.009*	0.981
Δ Potassium	0.002*	0.001*	0.893
Magnesium	0.007*	0.201	0.995
Δ Magnesium	0.031*	0.013*	0.856
GLP-1	0.268	0.000*	0.973
Δ GLP-1	0.316	0.000*	0.816
GIP	0.261	0.000*	0.964
Δ GIP	0.677	0.000*	0.952

Δ = change
Group-Time = bread group by time interaction
Values are p-value results of the analyses of variance by two-way ANOVA
* Statistical significance at the level of %5 (p-value < 0.05)

8. Glycemic Indices of Breads:

The area under the curve was calculated according to the trapezoidal rule in geometry. The GI rating (%) of the four bread types was then calculated by dividing the AUC for the test food (bread types) by the AUC for the reference food (white bread), and multiplying by 100. The average GI value was then calculated from data collected from all 12 subjects (Table 23). WB-F had significantly retained the lowest GI as compared to WB.

Table 23: Mean glycemic indices of all bread groups:

	WB	**WG**	**WB-R**	**WB-F**
Mean GI (n=12)	100.00±0.00	105.2±20.5	67.3±12.5	62.91±7.97[c]

Values are mean GI ± standard error of the mean

[a, b, c] Values are significantly different from control test food (WB) at $p<0.05$, $p<0.01$, and $p<0.001$ respectively, as analyzed by paired t-test.

CHAPTER V

DISCUSSION

Based on preliminary evidence suggesting that added cereal fiber has failed to induce a protective effect and was not inversely correlated with components of the metabolic syndrome, it was hypothesized in this study that the benefits of whole grains previously ascribed to their fiber content are in fact due to the minerals: phosphorus, potassium, and magnesium. This was also evident in previously published trials of wheat bran supplementation, which showed no improvement in conventional markers of glycemic control or risk factors for CHD in type II diabetes (Jenkins et al., 2002). It is worth noting that Jenkins et al. have only addressed the effects of wheat bran supplements, and not the provision of whole grains, which usually consists of other crucial and beneficial factors (germ, grain size). On the other hand, previous epidemiological studies and large cohort studies have evidently defined the metabolic benefits of whole grain cereals, and their protective role against the development of diabetes (Liu et al., 2000; Salmeron et al., 1997; Meyer et al., 2000), yet without being able to identify the exact marker or facet of wheat bran that is behind these beneficial effects (Pereira et al., 2002); which once again may be ascribed to the existence of these electrolytes. The proposed research thus aimed to restore and fortify white bread with these minerals and determine the resulting glycemic index of mineral-fortified bread.

The glycemic indices, or in other words the incremental area under the 2-hour blood glucose response curve following ingestion of WG, WB-R, and WB-F were 105.2, 67.3,

and 62.91 respectively, as compared to WB; WB-F in its turn significantly retained the lowest GI and ranked the lowest, indicating that white bread supplemented with the minerals phosphorus, potassium, and magnesium maintained a lower glycemic index and thus exhibited a better glycemic profile, as compared to non-supplemented breads (white bread and whole grain bread). Previous studies and authors have evidently demonstrated that low glycemic index diets are known to improve both lipidemic and glycemic profiles (Chiu et al., 2011; Goff et al., 2012). Hence, these physiological findings make it plausible to presume that fiber may be a marker of other components of whole grains that impart health advantages – in this case minerals content. In line with this, these electrolytes have been known to improve carbohydrate metabolism and exhibit beneficial effects on glycemic profiles (Haap et al., 2006; Belin and He, 2007; He and MacGregor, 2008; Lippi et al., 2009).

Participants of this study (n=12) had normal glycemic profiles; impaired glucose homeostasis was not identified since none of the subjects had increased levels of serum glucose (> 140 mg/dl), during the two hours post bread ingestion. Both serum glucose and insulin levels increased post ingestion of all four bread types. In accordance with several previous studies, glucose levels peaked at 30 minutes, and began to decrease at 60 minutes in all bread groups. At 60 minutes and on, WB-R and WB-F significantly retained the lowest glucose levels, further indicating that this metabolic effect is not attributed to fiber content but instead to that of minerals. At 120 minutes, WG, WB-R, and WB-F significantly maintained the lowest glucose levels, with WB-F being the lowest. Unlike WB which experienced the slowest serum glucose clearance, both WB-R and WB-F had a

significant rapid clearance of glucose from circulation starting 60 minutes. This metabolic effect verifies the essential role of phosphorus in carbohydrate metabolism, specifically via phosphorylation of glucose into glucose-6-phosphate (G-6-P) – the most vital step in glycolysis, necessary for glucose clearance and transport into cells (Bouche et al., 2004). It is also worth mentioning that throughout all time intervals, WG and WB-R followed an analogous trend, thereby suggesting a sort of resemblance in their composition.

Analogous to glucose, insulin in its turn peaked at 30 minutes in all bread groups except for WG , at 60 minutes. However, insulin levels did not show any statistical significance across the four bread groups, neither when compared to control test food (WB). Insulin findings of this study, are in line with the findings of several preceding epidemiological trials which have in turn revealed a direct relationship between decreased serum phosphate concentrations and insulin insensitivity and high postprandial blood glucose levels, while insulin levels remained unaltered (Haap et al., 2006; Friedman 2007). Khattab et al. also determined that phosphate supplementation was able to reduce both insulin and HOMA at 60 minutes, thereby improving insulin sensitivity after an oral glucose load. Furthermore, researchers demonstrated that phosphate supplementation among insulin insensitive hypophosphatemic patients markedly enhanced insulin sensitivity and glucose tolerance, independent of pancreatic insulin secretion (Wittmann & Nagy, 1997). This indeed implies that it is insulin sensitivity and not insulin secretion that is mainly affected by serum phosphate status. In this study, the combined results of both glucose and insulin, suggest that insulin sensitivity was improved in mineral supplemented breads (WB-R and WB-F). Both WB-R and WB-F revealed faster clearance and better

glucose tolerance as compared to non-supplemented breads (WB and WG), while insulin levels remained unaltered. A major limitation with regards to insulin response may be due to the fact that insulin was measured and analyzed at only two time intervals (30 and 60 minutes), thus limiting the chances of detecting significance across and within bread groups.

Throughout all time intervals, mean serum phosphate levels were higher in WB-R and WB-F as compared to WB and WG. Mean serum phosphate levels declined straight away after ingestion of all bread types. Previous trials have as well identified drop offs in post-prandial serum phosphate levels following consumption of a glucose load as a result of insulin secretion, which in turn stimulates glucose uptake into cells along with intracellular shift of phosphate to initiate glucose phosphorylation (Berthelay et al., 1984; Amanzadeh and Reilly, 2006). Nevertheless, in this study, the decrease in serum phosphate levels in WB-R and WB-F groups was later followed with an uninterrupted pick up at 45 minutes; unlike non-mineral supplemented breads WB and WG which experienced a continuous decrease in serum phosphate levels throughout the entire 2 hours. Serum phosphate levels in WB-R and WB-F experienced an increase starting 45 minutes, due to the fact that exogenous inorganic phosphate gets absorbed along the entire length of the intestine, and therefore needs around 45 to 60 minutes to get fully absorbed (Sabbagh et al., 2011). In parallel to these findings, a recent study has showed that the addition of 500 mg phosphorus to an oral glucose tolerance test (OGTT) was able to prevent the reduction in both total and inorganic phosphates, and in turn reduce insulin and HOMA starting 60 minutes and on (Khattab et al., 2011). Furthermore, Khattab et al., have also revealed that

ingestion of pure glucose is known to be associated with a decrease in postprandial plasma concentrations of the minerals phosphorus, potassium, and magnesium; and that their inclusion in a meal was reported to ameliorate this postprandial decrease.

Unpredictably, mean serum phosphate levels were significantly low post ingestion of WG bread all through the 2-hour postprandial period, whereby the WG serum phosphate curve continued to experience a steady decrease, and have ranked the lowest. Likewise, both WG serum potassium and WG serum magnesium curves revealed that postprandial serum levels of these corresponding minerals were notably low in response to consumption of WG bread. The low postprandial levels of these minerals (particularly of phosphorus) following the ingestion of WG bread is probably due to the presence of elevated phytate content in whole wheat flour, in addition to short fermentation periods. Phytic acid is the major storage form of phosphorus contained mainly in the germ and bran of most cereal grains (Harland and Harland, 1980). Thereby whole grain cereals are considered to be richest in phytate. Phytic acid is known for its strong chelating properties and its ability to bind to multivalent metal ions and other minerals, rendering them biologically unavailable (Estepa et al., 1999). Yeast, the major ingredient of baked products, contains a phosphatase which can hydrolyze phytate to orthophosphate and inositol, thus eliminating the available binding sites, and liberating the previously chelated minerals (Harland and Harland, 1980). When the dough is incubated at warm temperatures and fermentation gets initiated, the activated phosphatase hydrolyzes phytate, thereby increasing overall inorganic phosphate and minerals availability (Harland and Harland, 1980). Previous experiments on bread composition and analysis come together on confirming that phytate concentrations may be

effectively reduced by increasing yeast amount or prolonging fermentation and leavening time, ideally for up to 2 hours (Ranhotra et al., 1974; Harland and Harland, 1980; Estepa et al., 1999). One of the main limitations of this study is that all bread types (including WG) were incubated and leavened for 45 minutes only, which according to previous experiments is considered insufficient for releasing chelated minerals and increasing their bioavailability, which in turn explains our findings. The notable decline in serum phosphate levels post ingestion of WG - all through the 2-hour postprandial period, may possibly explain the elevated WG serum glucose and insulin curves; thereby suggesting, decreased glucose phosphorylation in response to insulin, and impaired cellular glucose uptake.

As for magnesium, statistical significance was detected between different time intervals and across bread groups. It is worth mentioning nevertheless, that the WG curve plotted the lowest amount of serum magnesium levels. Post ingestion of WB-F and WB-R (not significant), mean serum magnesium levels experienced a significant decrease, followed by an uninterrupted pick up at 45 minutes and 60 minutes in both bread groups respectively. Recent clinical studies have associated altered magnesium status as an independent risk factor for the development of metabolic syndrome and of each of its components (Larsson & Wolk, 2007; Song et al., 2005). A recent trial found that magnesium supplementation of a meal increases postprandial magnesium levels and improves hyperlipidaemia in otherwise healthy subjects (Kishimoto et al., 2010). In this study, postprandial triglycerides decreased significantly post ingestion of mineral fortified bread, hence magnesium‘s effect on triglycerides was markedly noticeable. Similarly the literature lists several trials in which magnesium was reported to reduce glucose tolerance

to an OGTT (Zofková et al., 1987), and improve glucose disposal rate (Yajnik et al., 1984), while insulin levels were not altered and this may relate to an improvement in insulin sensitivity, thereby enhancing both glucose and insulin metabolism (Swaminathan, 2003). These findings are in line with this study, whereby the mineral fortified breads WB-R and WB-F have experienced a highly significant and rapid clearance of glucose from circulation, as compared to WB which has had the slowest rate – while insulin levels remained unaltered.

Similar to magnesium, potassium curves showed significant differences within bread groups and across time intervals. As expected, the mineral fortified breads WB-F and WB-R (not significant) maintained a level of postprandial potassium that is significantly higher than that of WB at 45 minutes and on. The non-supplemented WB and WG bread groups however experienced a continuous gradual decrease in serum potassium levels. Usually, serum potassium concentration is tightly regulated via homeostatic mechanisms within the narrow range of 3.5 to 5.5 mEq/L (Osorio & Linas, 1998). Previous findings have showed that low potassium diets induced glucose intolerance that was associated with impaired insulin secretion, and was shown to be stimulated by potassium infusion (Rowe et al., 1980; Dluhy et al., 1972).). In line with this, a strong relationship between thiazide-induced hypokalemia and glucose intolerance was reported in thiazide treated subjects (Zillich et al., 2006). A recent study has also shown that experimentally induced hypokalemia have as well resulted in glucose intolerance due to a decline in pancreatic beta cells sensitivity to glucose loads, and thus low insulin secretion levels (Chatterjee et al., 2011). In this study however, no significant change was identified in insulin secretion

levels across all four bread groups; this may be due to the fact that all subjects started off having normal levels of serum potassium, and none was hypokalemic. Nevertheless, the mineral fortified breads WB-R and WB-F have experienced a highly significant and rapid clearance of glucose from circulation, as compared to un-supplemented breads.

Serum triglycerides have showed statistical difference across the four bread groups. As projected, serum triglycerides change curve showed that WB-R and WB-F retained the lowest triglycerides levels, as compared to WB. In line with this, previous trials have showed a direct correlation between serum phosphate and improved lipid profile whereby increased serum phosphate levels altered phospholipid metabolism and in turn increased HDL and decreased serum triglyceride levels (Haglin, 2001; Kalaitzidis et al., 2005; Park et al., 2009). This metabolic effect is due to the anabolic action of insulin in response to a glucose load, which in its turn stimulates lipoprotein lipase to activate cellular uptake and clearance of triglycerides (Vossen et al, 2011). Given that in this study postprandial triglycerides decreased significantly post ingestion of mineral fortified bread, insulin and phosphate‘s effect on triglycerides was markedly noticeable.

The incretins, GLP-1 and GIP were as well measured in response to mineral supplementation. An increase in GLP-1 and GIP concentrations was expected to take place post ingestion of the mineral supplemented bread. Researchers have linked adequate phosphate levels to increased incretin production, as a result of increased cAMP production (Baggio and Drucker, 2007). Increased cAMP concentrations activate the PKA pathway, which in turn stimulates proglucagon synthesis, and eventually GLP-1 synthesis (Baggio and Drucker, 2007). In this study however, neither GLP-1 nor GIP showed any statistical

difference between the four bread groups; however they were statistically different between the different time intervals (baseline, 30 and 60 minutes) within the same bread group. GLP-1 has peaked at 30 minutes post bread ingestion, while GIP has peaked at 60 minutes. Previous studies have showed that both incretins tend to slow down gastric emptying, improved fasting glucose levels, decreased postprandial glucose levels, improve pancreatic beta cells function, and prevented the postprandial increase in triglycerides (Meier et al., 2006; Baggio & Drucker, 2007; Kim & Egan, 2008). Although in earlier trials, phosphorus-rich meals have stimulated generation of incretins which have in turn improved glycemic profiles, insulin insensitivity, and dyslipidemia, the findings of this study failed to show any significant alterations in incretins concentration or insulin sensitivity. This may be due to the fact that GLP-1 and GIP have a very short half life of 2 minutes and 5 minutes respectively, after which they get rapidly cleaved by intrinsic dipeptidyl peptidase 4 (DPP-4), thus rendering them biologically inactive (Kim & Egan, 2008). Another related reason and a limitation to this research study, is that GLP-1 and GIP were not stabilized by protease inhibitors upon withdrawal of human plasma. Ex vivo stabilization of GLP-1 and GIP by protease inhibitors is imperative and crucial for their utility in clinical settings (Yi et al., 2010). Stabilization grants a basis for correct measurement of these metabolic peptides in diagnostic practices (Yi et al., 2010).

Put together, the identified limitations of this study include:

- Insulin was measured and analyzed at only two time intervals (30 and 60 minutes), limiting the chances of detecting significance across and within bread groups.
- Bread was incubated and leavened for 45 minutes only, which is considered insufficient time for releasing chelated minerals, thus rendering them biologically unavailable.
- GLP-1 and GIP did not undergo ex-vivo stabilization by protease inhibitors upon withdrawal of human plasma, and thus weren't accurately measured.

CHAPTER VI

CONCLUSION

This research study has successfully identified the potential beneficial role of minerals in improving glycemic index of white bread and overall glucose control in healthy male subjects. Even though, the beneficial effects of whole grains have been widely mediatized, yet the adoption of diets rich in whole grains still faces resistance. This may partially be influenced by the low palatability of unrefined cereal products. Therefore there is a need to develop palatable cereals with low glycemic index. White flour is the basis for staple foods in many countries and contributes significantly to high glycemic index diets. Thus, modifying the glycemic index of white flour, while maintaining its palatability, would be a practical approach to reducing the glycemic index of diets, and promoting further health advantages.

Results from the this study are promising and may prove to be the backbone for future research that investigates the long term effect of mineral-supplemented bread on glycemic status, and this may be used as a tool for the prevention or management of diabetes and other components of the metabolic syndrome. The ability of mineral supplementation to lower glycemic index would initiate research on: the relation between electrolyte intake and the development of glucose intolerance. Eventually, this would initiate research toward the establishment of a recommended mineral: carbohydrate ratio in food or mineral: energy ratio, similar to that of thiamin: carbohydrate or energy ratio.

APPENDIX

I. CONSENT FORM (ENGLISH)

Study Title: Glycemic index of bread: fiber or minerals? This is the question.

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Co-Investigator: Dr. Hala Ghattas/ Dr. Imad Toufeili / Dr. Ammar Olabi/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Dr. Sami Azar / Department of Internal Medicine, Division of Endocrinology and Metabolism, American University of Beirut, medical center/ American University of Beirut School of Medicine

Researchers: Dareen Shatila(Research Assistant), Rania El-Khoury (Graduate student)

Address: American University Beirut, Cairo Street, Hamra, Beirut – Lebanon/01 – 350000

Where the study will be conducted: Faculty of Agriculture and Food Sciences/Department of Nutrition or the Central research unit (CRU)/ American University of Beirut-medical Center

We are asking you to participate in a **research study**. Before agreeing to participate in the research, it is important that you read the information below. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures, if any, available to you, as well as your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

A. Purpose of the Research Study: The purpose of this study is to fortify white bread with phosphorus, potassium and magnesium and test the acceptability and the resulting increase in blood glucose levels. White flour will be fortified with minerals and bread will be prepared from the mineral-fortified flour. Subjects will be given 50 g of the bread, blood samples will be collected at different time intervals post-ingestion for glucose determination.

B. **Project/Procedures Description:** total of 12 healthy individuals will be recruited for this study. Participants who are interested in joining the study will be briefed about the study objectives and procedures by a trained research assistant. The participant will be provided with any additional information he/she might be inquiring about. Individuals who accept to participate in the study will be invited into an allocated private room in the American University of Beirut Medical Center and a written informed consent will be obtained from them, after they have read all the terms and conditions for participation. Data collection and interviews with participants will only take place at AUBMC. Participants who wish to be part of this study will have to come for 3 additional visits, following their first interview visit. The duration of each interview/visit would be around 3 hours. Participants will be asked to maintain their regular dietary and physical activity habits during the entire study course, avoid alcohol consumption as well as any unusual strenuous exercise 24 hours prior to the study. Following a 12 hour (overnight) fast, they will be taken to the testing facility [Faculty of Agriculture and Food Sciences/Department of Nutrition

or the Central research unit (CRU)/ American University Hospital] where: anthropometric measurements (height, weight) will be taken. A catheter will be inserted for blood withdrawal. In brief, overnight fasted subjects will be given 90g bread (45 g of carbohydrate) to ingest within 10-15 min and drink 250ml of water. Blood samples will be taken immediately before the meal and at 15, 30, 45, 60, 90 and 120 minutes after meal ingestion. Blood will be withdrawn by a trained practitioner and will be used for the determination of serum glucose levels. In addition, mineral content of the blood will be determined and several metabolites (triglycerides, phosphorus, potassium, magnesium) and hormones (insulin) will be measured. The results of the assessment will be communicated to you. Your participation in this research is completely voluntary but it is very important to us. By participating in this study, you will be helping us to investigate the effect of mineral supplemented bread on blood glucose levels, and this may be very useful for the prevention and management of diabetes as well as other diseases.

C. Duration: If you decide that you want to take part in this study, you will be kindly asked to allow us to re-contact you and re-invite you to AUBMC every 10 days for a one month period (after the initial interview), in order to redo the same procedure with 3 other types of bread.

D. **Risks, Discomforts and Benefits:** There are no expected risks to you for helping us with this study. There are no expected direct benefits either. Also note that refusal to participate in this study will involve no loss in benefits. If you agree to participate in this research study, the information will be kept confidential under lock and key. In addition, all of your healthcare providers including your physician will not have access to the information you provided in this study.

E. **Confidentiality:** Efforts will be made to keep your child's study-related information confidential. All data from this study will be maintained in a secure locked drawer in a locked office or on a password protected computer. Data will only be reported in the aggregate. No names of individual children will be disclosed in any reports or presentations of this research. However, there may be circumstances where this information must be released. For example, personal information regarding your child's participation in this study may be disclosed if required by law. Also, your child's research data may be reviewed by the following groups (as applicable to the research):

- U.S. Office for Human Research Protections or other federal, state, or international regulatory agencies, required;
- The AUB Institutional Review Board or Office of Human Research Protections;
- The sponsor, if any, or agency supporting the study.

After the conclusion of the study, the Principal Investigator will retain all original study data in a secure location for at least three years to meet institutional archiving requirements. After this period, data will be responsibly destroyed.

F. Compensation/Incentive: No costs have to be paid by you. There will neither be anticipated expenses for participating and costs for transportation, parking etc will not be reimbursed.

G. Payment for Research-related Injury: In case of any adverse event as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by a third party or governmental insurance.

If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

H. Contact Information and Questions:

1) If you have any questions or concerns about the research you may contact: Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb.

2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact the following office at AUB: Social & Behavioral Sciences Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440 or 5445

J. Future Contact

Would you like to be contacted for future research? Yes _____ No _____

Please notify that the investigator has the right to end subject's participation in this study.

Participant Consent:

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to give permission for my minor child (or child under my guardianship) to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to give permission for my child/child under my guardianship to participate in this study. I am not giving up any legal rights by signing this form. I will be given a copy of this form.

_____

Printed name of subject

_____ AM/PM

Date and time

Investigator/Research Staff

I have explained the research to the parent or legal guardian of the child subject/participant before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the parent/legal guardian of the child participant/subject.

_____ _____

Printed name of person obtaining permission Signature of person obtaining permission

_____ AM/PM

Date and time

APPENDIX

II. CONSENT FORM (ARABIC)

موافقة للإشتراك في البحث العلمي

عنوان البحث: مؤشر نسبة السكر في الدم من الخبز: الألياف أو المعادن؟ هذا هو السؤال.

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين:د. هلا غطاس/د.عماد طفيلي/د.عمار علبي/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

د.سامي عازار/ قسم الطب الباطني/ شعبة الغدد الصماء والأيض/ الجامعة الأميركية في بيروت- المركز الطبي / الجامعة الأميركية في بيروت - كلية الطب.

منسقي البحث: دارين شاتيلا، رانيا الخوري.

العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان01-350000

مكان إجراء البحث: كلية الزراعة والعلوم الغذائية / قسم التغذية أو وحدة البحوث المركزية (CRU) / الجامعة الأميركية في بيروت – المركز الطبي

أنت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في الجامعة الأميريكية في بيروت. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الإستمارة أو عن هذه الدراسة ككل من طبيبك.

أ) هدف هذا البحث: الغرض من هذه الدراسة هو لتدعيم الخبز الأبيض بالبوتاسيوم، والفوسفور والمغنيسيوم واختبار مدى تقبل الطعم و اختبار الزيادة الناتجة في مستويات السكر في الدم. سيتم تدعيم الطحين الأبيض بالمعادن وسيتم إعداد الخبز الابيض من هذا الطحين المدعم بالمعادن. وسيطعم 50غ من الخبز للمشاركين، سيتم جمع عينات الدم في فترات زمنية مختلفة بعد تناول الخبز لتحديد كمية الجلوكوز.

ب) **وصف الإجراءات والمشروع:**سيتم تعيين اختيار 12 فردا يتمتعون بصحة جيدة للمشاركة في هذه الدراسة. وسيتم إطلاع المشاركين الذين يرغبون في الانضمام إلى هذه الدراسة حول أهداف الدراسة

والإجراءات من قبل مساعد باحث. وسيتم توفيرللمشارك أي معلومات إضافية له / لها اذا اراد الاستفتار عن أي شئ. سيدعى الأفراد الذين يقبلون المشاركة في الدراسة الى غرفة مخصصة في الجامعة الأميركية في بيروت -المركز الطبي وسيتم الحصول على موافقة خطية مستنيرة منهم، بعد قراءة جميع البنود وشروط المشاركة. جمع البيانات وإجراء مقابلات مع المشاركين سوف يتم في الجامعة الأميركية في بيروت – المركز الطبي فقط .وسيقوم المشاركون الذين يرغبون أن يكونوا جزءا من هذه الدراسة يجب أن يأتوا لمدة 3 مرات إضافية، بعد زيارتهم للمقابلة الأولى. ومدة كل مقابلة / زيارة حوالي 3 ساعات.
وسيطلب من المشاركين الحفاظ على عاداتهم اليومية من النشاط البدني والنظام الغذائي أثناء الدراسة بأكملها، وتجنب استهلاك الكحول وكذلك أي ممارسة غير عادية مضنية قبل 24 ساعة من الدراسة. بعد 12 ساعة من الصوم سيتم نقلهم إلى مرفق اختبار [كلية الزراعة والعلوم الغذائية / قسم التغذية أو وحدة البحوث المركزية (CRU) / الجامعة الأميركية في بيروت – المركز الطبي] حيث سيتم اتخاذ القياسات (الطول والوزن).
وسيتم إدخال الابرة لسحب الدم. باختصار، سوف يتم اعطاء المشاركين على الريق الخبز (45 غرام من الكربوهيدرات) و ml250 كوب من الماء لتناوله خلال 10-15 دقيقة. وسيتم أخذ عينات الدم مباشرة قبل الوجبة وبعد 15، 30، 45، 60، 90 و 120 دقيقة بعد تناول الوجبة. وسيتم سحب الدم عن طريق مختصين وسيتم استخدامها لتحديد مستويات الجلوكوز في مصل الدم. وسيتم قياس الدهون الثلاثية، والفوسفور، والبوتاسيوم، والمغنيسيوم والهرمونات (الأنسولين). وسيتم إبلاغ نتائج التقييم لك. مشاركتكم في هذا البحث هو طوعي تماما ولكن من المهم جدا بالنسبة لنا. من خلال المشاركة في هذه الدراسة، سيتم مساعدتنا على دراسة تأثير الخبزالمدعم بالمعادن على مستويات السكر في الدم، وهذا قد يكون مفيدا جدا للوقاية من مرض السكري و الأمراض الأخرى.

ج) **المدة**: إذا قررت المشاركة في هذه الدراسة، سوف نتمكن من إعادة الاتصال بك دعوتك إلى **الجامعة الأميركية في بيروت – المركز الطبي** كل 10 أيام لمدة شهر واحد (بعد المقابلة الأولية)، من أجل اعادة الإجراءات ذاتها مع 3 أنواع أخرى من الخبز.
د) المخاطر والمضايقات والفوائد: لا يوجد مخاطر من جراء مساعدتنا في هذه الدراسة. ولا توجد فوائد مباشرة ايضا. يمكنك الانسحاب من البحث في أي وقت. إن أردت التوقف عن المشاركة، ما من عقوبة تفرض عليك ولن تخسر أي من الفوائد التي تملكها وقرارك لن يؤثر على أي علاقة مستقبلية مع الجامعة الأمريكية في بيروت.. إذا كنت توافق على المشاركة في هذه الدراسة البحثية، سيتم حفظ كل المعلومات بسرية بموجب القفل. وبالإضافة إلى ذلك، كل من مقدمي الرعاية الصحية الخاص بك بما في ذلك الطبيب لن يكون لديه الصلاحية للوصول إلى المعلومات التي قدمتها في هذه الدراسة.

و) **التعويض / الحافزة:** ليس هناك أية تكاليف مطلوبة منك أن تدفعها و لن تتقاضى أي أجر لهذه الدراسة، ولن تتقاضى أجر التنقل أو كلفة موقف السيارة الخ.

ز) **الدفع للإصابات ذات صلة بالبحث:** ما من تغطية لحصول الحوادث الغير متوقعة، في حال لم تكن هناك تغطية من قبل تأمين صحي أ, ضمان اجتماعي. إن تعرضت إلى إصابة جراء البحث، أو لأي سؤال عن الإصابات

المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد 350000 (01) مقسم 4440، email: oo01@aub.edu.lb

ح) **أسئلة ومعلومات الاتصال**
١) لأي أسئلة أو أي مخاوف حول البحث، يمكنك الاتصال بالدكتور عمر عبيد، قسم التغذية وعلم الطعام الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان 350000 (01) مقسم 4440، email: oo01@aub.edu.lb.
٢) لأي أسئلة أو أي مخاوف حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية: irb@aub.edu.lb

ح) **حقوق المشاركين:** مشاركتك في هذا البحث طوعية. يمكنك مغادرة البحث في أي وقت من دون أي عقوبة. إن قرارك بعدم المشاركة لن يؤثر بأي شكل ممكن على علاقتك بالجامعة الأمريكية في بيروت.إذا اخترت الاشتراك في هذه الدراسة، يمكنك وقف اشتراكه فى اى وقت بدون عقوبة او فقدان الاستحقاقات. بتوقيع هذا البيان, لن تتخلى عن اى حقوق قانونية أو شخصية اذا قمت أنت أو طفلك بالمشاركة فى هذه الدراسة.مجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت المسؤولة عن الابحاث التي تتعلق بالبشر قد قامت باستعراض هذا المشروع البحثى,و وجدت أنه مقبول حسب القضاء اللبناني و اللوائح الفيدرالية في الولايات المتحدة الأمريكية . إن الجامعة الامريكية لها سياسات تهدف الى حماية حقوق ورفاهية المشاركين فى البحث.

أ. **<u>الاتصال في المستقبل:</u>**
هل ترغب في الاتصال بك للمشاركة في أبحاث أخرى في المستقبل؟ نعم _____ لا _____
ملاحظة: للباحث الحق الكامل بايقاف أي مشارك عن متابعة مشاركته في هذا البحث.

موافقة المشترك: لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء عليه فأنني، حرا مختارا، أجيز الموافقة لابني أو ابنتي تحت وصيتي إجراء هذا البحث و أوافق أن يشارك فيه . لن أتخلى عن أية حقوق قانونية عند امضائي لهذا البيان كما أنني سأستلم نسخة من هذا البيان.

إسم المشترك _____ التاريخ_____توقيع المشترك_____

الباحثون: لقد شرحت كل التفاصيل التي تتعلق بهذا البحث لأهل الطفل المشارك أو للوصي الشرعي قبل الحصول على امضاء الأخير. لا يوجد فراغات في هذه الوثيقةز و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

الإسم المطبوع للشخص الذي يطلب الموافقة:_____

إمضاء الشخص الذي يطلب الموافقة:_____

التاريخ و الوقت:_____

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