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## Abstract

**Background:** Metabolism of refined carbohydrates, which are associated with detrimental health effects, is known to be affected by macrominerals including P, Mg and K.

**Aims:** To assess the impact of their addition to flour on the sensory properties of white pita bread and postprandial glycaemia of healthy individuals.

**Methods:** The study was conducted at the American University of Beirut (between February and October 2014). Plain, restored and fortified wheat flour, with macrominerals were used to prepare 3 types of bread: white pita bread (WP), restored white pita bread (WP-R) (pre-milling levels) and fortified white pita bread (WP-F) (double the pre-milling levels). Sensory characteristics of bread were assessed and postprandial glycaemia was determined using a single-blinded crossover design whereby participants consumed 1 of the 3 different types of pita bread in random order.

**Results:** No significant difference ( $P > 0.05$ ) between the different types of bread was detected using the triangle and acceptability tests, except for texture ( $P < 0.05$ ). Macromineral enrichment of bread (WP-R and WP-F) significantly reduced postprandial glucose ( $P = 0.013$ ) and triglyceride ( $P = 0.001$ ) levels.

**Conclusions:** Macromineral enrichment of refined carbohydrates may have a promising role in

lowering postprandial glucose and triglycerides, and thus decrease their negative health consequences.

Keywords: sensory properties, glucose, triglyceride, white bread, macromineral enrichment

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## Introduction

Over the past few decades, there have gradual but significant changes in eating behaviour worldwide in light of nutritional transition from traditional diets rich in complex carbohydrates to diets high in simple carbohydrates (1). These changes were associated with an increase in the prevalence of chronic diseases and accordingly, the recent dietary guidelines in the United States of America (2) strongly emphasize the importance of reducing simple carbohydrates. Fibre, vitamin and mineral content of flour is drastically reduced by conventional milling and grain refinement processes. Macrominerals including P, Mg and K, are reduced by about 69%, 74% and 84%, respectively (3), and are known to improve postprandial glucose and insulin metabolism (4,5). P plays an essential role in carbohydrate metabolism via phosphorylation of glucose to glucose-6-phosphate; an essential step for glucose clearance and trapping into cells (6). The need for P is highest during the postprandial period, as indicated by its reduced level after glucose ingestion and by the improvement in insulin sensitivity following its addition (7). Mg mediates glucose transport mechanisms into the cell membranes through its effect on insulin signalling via tyrosine kinase activity, phosphorylase B kinase activity and glucose transporter protein activity (8,9). K in its turn acts as a cofactor for several enzymes involved in carbohydrate phosphorylation and oxidation, such as protein kinases and phosphatases. K is

also known to affect glucose tolerance (5).

Thus, low availability of the above-mentioned macrominerals would be expected to delay postprandial cellular uptake of glucose, impair phosphorylation, and eventually hinder carbohydrate metabolism and energy production (10). These conditions would ultimately favour the onset and development of the different components of metabolic syndrome, especially impaired glucose tolerance and diabetes (11). In this same context, diminished insulin sensitivity is known to promote hypertriglyceridaemia (12); therefore, serum triglyceride (TG) levels are expected to increase in a setting of low mineral availability.

Refined white flour has received extensive worldwide acceptance, since it is used to produce baked goods that are more palatable, softer in texture and have extended freshness. White pita bread is heavily consumed in the Middle East and increasingly in Europe and North America. Therefore, the objective of this work was to assess the impact of P, Mg and K enrichment on sensory properties and postprandial glycaemia of white pita bread.

## **Methods**

### **Study design**

This study was conducted between February and October 2014, according to the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board at the American University of Beirut (approval no. NUT0019). Written informed consent was obtained from all participants. The clinical trial was registered with Clinical Trial.gov, NCT02598986.

Wheat flour (80% extraction; Bakalian Flour Mills, Beirut, Lebanon) was used and 2 levels of mineral supplementation were made. Restoration: minerals were added to white flour so that each kilogram contained 3.6 g MgCO<sub>3</sub> (G&G Vitamins, East Grinstead, UK) and 12.5 g KH<sub>2</sub>PO<sub>4</sub> (Dyets, Bethlehem, PA, USA). Fortification: minerals were added to white flour to almost double the original levels, so that each kilogram of white flour contained 7.2 g MgCO<sub>3</sub> and 25 g KH<sub>2</sub>PO<sub>4</sub>. The amounts of added P and Mg were considered safe since both were lower than the tolerable upper limits set at 4 g/day and 350 mg/day for P and Mg, respectively (13). After supplementation, different types of white pita bread were made and used for the different tests.

### **White pita bread making**

Bread samples were prepared as previously described (14). Upon termination of the bread

making process, 3 samples from each type of bread [white pita bread (WP), white pita bread-restored (WP-R) and white pita bread-fortified (WP-F)] were analysed for their mineral content by inductively coupled plasma mass spectrometry (ICP-MS) using the standard method EPA 200 – 7/8 (15). P, Mg and K contents of WP-R were 84%, 200% and 60% higher than those of WP, respectively ( [Table 1](#) ). P, Mg and K contents of WP-F were 260%, 410% and 230% higher than those of WP, respectively. P, Mg and K contents of WP-F were almost double those of the WP-R.

### **Experiment 1: difference and acceptability sensory tests**

Twenty-four healthy untrained male volunteers participated in a difference/discrimination test. Two triangular tests were conducted to compare WP versus WP-R or WP-F. Panellists were asked to indicate the odd sample in each set and to rinse their mouths before each sample. A consumer acceptability test was conducted with 60 healthy randomly recruited panellists (29 women and 31 men, mean age 22 years, range 19–29 years) from the American University of Beirut as described previously (16). The 3 samples used in different tests were assessed. Ten grams of each type of white pita bread were prepared 2 hours prior to serving them and were stored in the refrigerator (4°C). Panellists rated overall acceptability, appearance, colour, odour, flavour and texture on a 9-point hedonic scale (17). Panellists were instructed to rinse their mouths before each sample. The order of the samples within each set was randomized among the panellists in both tests.

### **Experiment 2: determination of postprandial glucose and triglyceride**

Independently from the first experiment, 11 healthy male volunteers were recruited and asked to maintain their regular dietary habits and physical activity during the entire study course, and to avoid alcohol consumption and unusual strenuous exercise 24 hours prior to each experimental session. Volunteers were aged 18–30 years (mean 24.5 years) with body mass index between 18.5 and 29.9 kg/m<sup>2</sup>, without significant medical or chronic diseases, with no regular use of medication that affected body weight, and without weight loss of  $\geq$  3% in the preceding 3 months.

A single-blinded, randomized crossover study was conducted. Each participant consumed 1 of the 3 different types of pita bread on each of 3 visits. The order of meals was assigned randomly and the visits were separated by a minimum washout period of 10 days. In each session, overnight fasted participants were asked to ingest 90 g (containing 50 g carbohydrate) white pita bread within 10–15 minutes and subsequently drink 200 ml water. Blood samples were collected at baseline (before ingestion) and at 15, 30, 45, 60, 90 and 120 minutes after ingestion. Blood samples were centrifuged for 15 minutes at 4°C at 2500 g and serum was stored in aliquots at  $-80^{\circ}$ C until analysis. Serum glucose, TG, and total P, Mg and K were measured using the Vitros 350 Chemistry System (Ortho-Clinical Diagnostics, Johnson & Johnson, New York, USA). Fasting serum insulin was determined using an ELISA kit (Diametra

Millipore Corporation, Billerica, MA, USA).

## Statistical analysis

Experiment 1: data related to triangular tests were analysed by checking the minimum number of correct responses using a binomial table with  $P = 0.05$  (17). As for the acceptability test, 2-way analysis of variance using the GLM procedure of SAS (version 9.02) was performed as described previously (16). In the statistical model for acceptability, the response variable was the specific acceptability variable. Factors in the model were the panellist and treatment (WP, WP-R and WP-F). The panellist was included as a random effect and treatment as a fixed effect. Means were separated by Tukey's honestly significant difference test. For all data, significance was established at  $P < 0.05$ .

Experiment 2: The difference ( $\Delta$ ) in serum total P, Mg, K, TG and glucose was calculated. This represents the value at each time point minus the value at time 0. Repeated-measures analysis of variance was used to determine statistical significance with effects of bread type, time, and bread type  $\times$  time interaction.

## Results

### Experiment 1

#### Difference test and hedonic acceptability

In the triangular difference test, 13 correct answers out of the 24 responses were needed to show a significant difference. However, only 8 and 10 panellists responded correctly for the WP versus WP-R and WP versus WP-F tests, respectively (both  $P > 0.05$ ). Therefore, the triangular tests did not detect any significant differences between the different types of bread.

The consumer acceptability test ( [Table 2](#) ) found no significant differences for most acceptability attributes (overall acceptability, appearance, colour, odour and flavour;  $P > 0.05$ ). Texture, however, was significantly more liked than that of the WP-F bread ( $P < 0.05$ ), although no significant difference was detected between WP and WP-R or WP-R and WP-F bread.

### Experiment 2

#### Participants' characteristics

Baseline fasting serum levels of glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR) (18), TG and total P, K and Mg were within the normal ranges ( [Table 3](#) ), and these were found to be similar between the different experimental sessions for each type of bread.

## Postprandial mineral responses

Results were expressed as changes from baseline, which were the difference between the macromineral levels at each time point minus their corresponding values at baseline. Postprandial serum levels of the measured macrominerals were altered by food ingestion. Serum total P decreased following ingestion of all bread types, although this failed to reach statistical significance. However, the changes in serum total P were significant between bread types ( $P = 0.015$ ), and serum P in WP-F bread returned to baseline by the end of the session ( [Figure 1A](#) ). Postprandial serum Mg levels experienced a gradual and significant increase with time ( $P = 0.027$ ), although no significant difference was detected among the different bread types ( [Figure 1B](#) ), despite their varied content of Mg ( [Table 1](#) ). In contrast to Mg, postprandial K levels decreased with time, although not significantly ( [Figure 1C](#) ), and were significantly different among bread types ( $P = 0.001$ ).

## Postprandial TG and glucose responses

Results of TG and glucose were also expressed as differences from baseline. The changes in postprandial serum TG ( [Figure 2A](#) ) differed significantly among bread types ( $P = 0.001$ ), and WP-R and WP-F maintained lower levels at all time points. Similarly, changes in postprandial serum glucose ( [Figure 2B](#) ) differed significantly among bread types ( $P < 0.013$ ) and over time ( $P < 0.001$ ). Serum glucose levels peaked at 30–45 minutes after ingestion and the peaks were sooner with the enriched pita breads. Thereafter, the enriched breads exhibited a faster decrease in serum glucose as compared to the WP bread, starting from 45 minutes until the end of the experiment. The magnitude of the decrease seemed to be synergistically related to the mineral content of the bread.

## Discussion

This study was designed to investigate the glycaemic response of macronutrient-enriched pita bread, as well as its sensory properties. Our results showed that the palatability of white pita bread was not affected by the addition of macrominerals, as indicated by the lack of differences in the triangular and acceptability tests. However, a small difference in texture was detected between WP and WP-F but not the triangular test, which is known to be more attentive to differences. Hence, no major differences were observed when the bread was assessed in its entirety. Our findings are in line with other studies, in which addition of K, Ca and Mg salts as replacements for NaCl did not yield any differences in appearance, texture and taste of brown bread (19). Therefore, it can be concluded that the addition of macrominerals to white wheat flour in an amount comparable to that found in whole wheat flour, and even in double quantities, does not significantly affect acceptability of white pita bread.

The reduction in serum P following ingestion of the different types of bread was in line with other studies (7,20), and this is mediated by insulin, which is known to stimulate peripheral uptake of both glucose and P. Thus, insulin favours glucose phosphorylation (21) in a manner that mimics the action of glucokinase activators (22). The inability of WP and WP-R to normalize serum P (return to baseline at 120 minutes) unlike that of WP-F (7) implies that their P content was not sufficient to meet the needs of intracellular phosphorylation. Moreover, the observed nonsynergistic relation between Mg content of the bread and changes in postprandial serum Mg is likely to result from the ability of P to potentiate insulin sensitivity (7,23,24), which is known to stimulate Mg clearance (20). Furthermore, improvement in insulin sensitivity may have also been attributed to the nonsynergistic relation between K content of bread and changes in postprandial serum K levels. Likewise, Mg (25,26) and K (27) are reported to improve glucose clearance and insulin sensitivity. The ability of macrominerals (P, Mg and K) to enhance their own intracellular uptake may help to explain the reported inverse association between P intake and blood pressure (28). This further implies that postprandial levels of these macrominerals depend on a balance between their availability in the circulation and their capacity for intracellular uptake and storage, and this implies that their circulating level is not a good indicator of their bodily status.

At the glycaemic level, the inverse association between the macromineral content of bread and postprandial glucose level, especially from time 60 minutes (7), may have been the outcome of an improvement in glucose clearance due to the capacity of the added minerals to improve glucose phosphorylation and insulin sensitivity. Furthermore, this capacity may have also contributed to the observed reduction in serum TG after ingestion of enriched bread (WP-R and WP-F). Our findings suggest that postprandial glucose and TG levels, especially from 60 minutes, are dependent on exogenous factors including P, Mg and K. In support, P status was reported to correlate with a favourable lipid profile, including increased high-density lipoprotein and decreased serum TG levels (10,12). In agreement, we have recently found that the addition of P to a high-fat meal was able to alter postprandial lipidaemia by increasing apolipoprotein B48 and decreasing apolipoprotein B100 (29). Besides, Mg supplementation also improves postprandial lipidaemic response in healthy individuals (30).

Worldwide, daily consumption of wheat and wheat products, mainly in the form of bread and pasta, is about 180 g per capita and this contributes to about 20% of total energy intake (31). Even with a consumption of 500 g/day of WP-R, which is considered an excessive amount compared to the reported daily consumption of about 150 g per day (32) and is equivalent to around 4604.6 kJ (1100 kcal), the upper limit for both P and Mg would not be reached. The high palatability of white pita bread makes it popular and a major contributor to overall glycaemic load, which increases the risk of development of diabetes, abnormal lipid profile and obesity (33,34).

Nonetheless, the health benefits of whole wheat cereal products are reported not to be related to their fibre content (35); therefore, our findings may partially explain the benefits of whole wheat products that are known to have high content of macrominerals, specifically P, Mg and K. Even though the beneficial effects of whole grains have been widely publicized, the adoption of diets rich in whole grains is still facing resistance, probably due to their low palatability.

The major limitation of this study was that postprandial insulin and other appetite hormone levels were not measured. In addition, the contribution of each mineral to the observed changes was not clear. Further studies are required to determine the postprandial response of prediabetic and diabetic patients, as well as the long-term impact of macronutrient enrichment on diabetes and different components of the metabolic syndrome.

## Conclusion

White wheat flour enrichment with macrominerals (P, Mg and K) did not affect the palatability of white pita bread, while postprandial glucose and TG levels were reduced. Furthermore, this supports the benefit of increasing the consumption of whole grain wheat, since it retains most of its mineral content. This study successfully identified the beneficial role of minerals in improving the glycaemic response of a simple carbohydrate product, white pita bread. The data may prove useful to ameliorate the detrimental potential effect of simple carbohydrates.

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Competing interests: None declared.

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