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Basic nutritional investigation

Concomitant lysine and phosphorus addition to a wheat gluten protein diet highly amplified growth measures of rats

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ABSTRACT

Objective: In humans, the effects of lysine-fortified wheat on growth measures was much lower than that of animal experimentations that used phosphorus-containing mineral mix. It is known that wheat contains a limited amount of available phosphorus, which is believed to support growth. The aim of this study was to determine the involvement of phosphorus in growth measures of rats maintained on a lysine-supplemented wheat gluten diet.
Methods: Forty male Sprague-Dawley (6 wk old) rats were randomly divided into four equal groups and fed wheat gluten protein (10%)–based diets with added lysine (0.6%), phosphorus (0.3%), or both (0.6% lysine and 0.3% phosphorus), ad libitum for 9 wk. Rats were monitored for changes in food intake, body weight, body and liver compositions, plasma urea nitrogen, and albumin.
Results: The addition of lysine or phosphorus to wheat gluten-based diets increased energy intake modestly (~15%), whereas their combination caused a higher increase (~45%). Similarly, the magnitude of improvement in weight gain and energy efficiency by the addition of lysine or phosphorus (~1g/d and 2.7g/MJ, respectively) was much lower than that of the combination (~4g/d and 8.7g/MJ). In the phosphorus-containing groups, plasma urea nitrogen was significantly reduced and this was associated with higher body protein (%) and hepatic fat (%); whereas plasma albumin was significantly increased in the lysine-containing groups.
Conclusion: When using gluten protein, concomitant lysine and phosphorus availability is required to support growth measures, although phosphorus seems to have an independent effect on protein metabolism. Thus, human interventions should consider the improvement of the amino acid profile and phosphorus availability.

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Introduction

Considerable variations exist in the quantity and quality of ingested proteins among populations. In developing countries, and for individuals following a vegetarian lifestyle, proteins are mainly derived from plant sources, with cereals, chiefly wheat and rice, being the major sources of protein and energy [1–3]. Wheat and its derived products are known to contribute to a large part of both the protein and energy supply [4]. In low-income countries, a significant number of people depend on cereals as their main or even sole source of both protein and energy. Because it is limited primarily in lysine and to a lesser extent in threonine [1,5], wheat protein gluten is of low quality. According to the World Health Organization/Food and Agriculture Organization [6,7], wheat has a low protein score of around 40 compared with about 100 for complete proteins (eggs, meat, and milk). Dietary restriction of lysine has been reported to cause growth retardation and feed inefficiency in rats and pigs [8–12]. In contrast, the addition of lysine to wheat gluten-based diets has been shown to improve the growth of weanling rats [13] and pigs [12]. Furthermore, supplementing a wheat gluten diet by as little as 5% casein was sufficient to arrest the decrease in feed efficiency, food intake, and weight gain of rats [14]. It is generally accepted that growth impairment precipitated by wheat gluten-based diets is reversed by either the addition of lysine or the ingestion of other complementary proteins with higher levels of the limiting amino acid [2,3,14,15]. In humans, unexpectedly, increased lysine intake, from fortified wheat flour products or ingestion of lysine, was reported to have marginal effects on the growth and maintenance of infants and children [2,3,15,16] and this raises a question about the involvement of other factors.

Omar Obeid conceived and designed the experiment. Marie-Elizabeth Ragi and Carla El Mallah performed the experiments and conducted the laboratory analysis. Marie-Elizabeth Ragi and Omar Obeid wrote the paper. All authors analyzed the data and read and approved the final manuscript. The authors have no conflicts of interest to declare.

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In wheat, the essential mineral phosphorus is mainly found in the form of phytate, which is not bioavailable [17,18]. In addition, some food preparation methods were reported to minimally improve bioavailability, for example, leavening (yeast fermentation) of wheat bread for ≤5 h increases soluble phosphorus by ~5% [19]. Phosphorus is involved in many metabolic reactions, including adenosine triphosphate generation, and thus is required for proper growth and maintenance [18,20–24]. Phosphorus-depleted diets have been shown to result in decreased growth rate, energy consumption, and energy efficiency in rats [25,26]. We recently found that the addition of bioavailable phosphorus was able to mitigate the deleterious effects of low-protein diets on the growth of rats [26].

The discrepancies in the growth effects of lysine addition to wheat gluten diets between human and animal studies encouraged us to investigate the possible involvement of phosphorus, especially because animal experimentations usually have sufficient quantities of bioavailable phosphorus from the mineral mix, unlike in most human experiments [9–11]. Thus, a study was designed to determine whether the growth measures of lysine-fortified wheat gluten protein is interconnected with phosphorus bioavailability.

Methods

Animal housing

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the American University of Beirut (AUB), Lebanon. The study was performed in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals. Forty male Sprague-Dawley rats (Animal Care Facility, American University of Beirut) weighing ~220 g (~6 wk old) were housed individually in wire-bottomed cages in a temperature (22 ± 1°C) and light-controlled room (12:12 h light/dark cycle, light on at 0700). The rats had free access to water and were fed a semisynthetic powder control diet ad libitum (Supplementary Table 1) for 1 wk to familiarize them with the environment and the diet.

Experimental diet

All experimental diets were prepared using the same ingredients with wheat gluten protein at 10% w/w, to mimic its level in wheat flour, as the only source of protein (Table 1). The groups' diets were varied in their contents of lysine and phosphorus as follows:

- Group G: gluten diet with no supplemental lysine or phosphorus.
- Group G+L: gluten diet with added lysine (0.6% w/w).
- Group G+P: gluten diet with added phosphorus (0.3% w/w).
- Group G+L+P: gluten diet with both lysine (0.6% w/w) and phosphorus (0.3% w/w).

Phosphorus-free mineral mix (AIN-93G MIX without phosphorus) and potassium phosphate (KH_2PO_4; Dyets Inc., Bethlehem, PA, USA) were used to manipulate the phosphorus contents of the diets. KH_2PO_4 was used as the phosphorus source because potassium does not affect the growth of laboratory rats [27,28]. In the phosphorus-supplemented diets, phosphorus was added at the level of 0.3% (3 g/kg) based on the AIN-93 recommendation for optimal growth of laboratory rats [29], whereas L-lysine HCl was added at 0.6% to cover the needs of growing rats (9.2 g/kg) [30].

Experimental design

In the present experiment, 6-wk-old rats were studied because they are still in the growing phase, after weaning, in order to study the growth parameters. Following the 1-wk adaptation period, the rats were randomly divided into four experimental groups (10 rats per group) and given their respective diets. Rats were fed ad libitum for 9 wk, then fasted overnight and anesthetized with isoflurane (Forane, Abbott, Berks, UK). Blood was collected from the superior vena cava. Thereafter, rats were sacrificed by severing their hearts. Their livers were immediately excised, weighed, frozen in liquid nitrogen, and stored at −80°C. Carcasses were stored at −20°C until analyzed for water and fat contents. Blood samples were centrifuged at 2200*g* (3°C) for 15 min, and aliquots of plasma were collected and stored at −80°C until analyzed.

Table 1
Dietary composition of different experimental groups of rats

The composition of the diet (g/kg)	Group G	Group G+L	Group G+P	Group G+L+P
Wheat gluten*,†	130	130	130	130
Cornstarch	345	342	338.4	335.4
Sucrose	345	342	338.4	335.4
Corn oil	100	100	100	100
Cellulose*	35	35	35	35
Mineral mix*,‡	35	35	35	35
Vitamin mix*,§	10	10	10	10
Potassium phosphate*,‖	–	–	13.2	13.2
L-Lysine HCl*,¶	–	6	–	6
The nutrient composition as % of total energy				
Protein	9.6	10.1	9.7	10.2
Carbohydrates	68.5	68.0	68.2	67.7
Fat	21.9	21.9	22.1	22.1
Total lysine and phosphorus content of the diets (g/kg)				
Lysine	3.8	8.6	3.8	8.6
Phosphorus	0.2	0.2	3.2	3.2
Total energy (MJ/kg)	17.49	17.49	17.27	17.27

G, gluten protein (10%w/w); G+L, gluten protein (10%w/w) and lysine (0.6%w/w); G+P, gluten protein (10%w/w) and phosphorus (0.3%w/w); G+L+P, gluten protein (10%w/w), lysine (0.6%w/w) and phosphorus (0.3%w/w).
*Obtained from Dyets Inc., Bethlehem, Pennsylvania, USA.
†The diets contained 10% protein coming exclusively from wheat gluten (76% protein).
‡"Phosphorus-free" mineral mix (AIN-93G mineral mix phosphorus-free, used as 35 g/kg of diet).
§Vitamin mix (AIN-93VX vitamin mix, used as 10 g/kg of diet).
‖Potassium phosphate KH_2PO_4, molecular weight 136 g/mol of which phosphorus 31 g/mol.
¶L-Lysine HCl, molecular weight 182 g/mol of which lysine 146 g/mol.

Food intake, body weight, and composition

Food intake and body weight were measured twice per week, and energy efficiency was calculated as weight gained per MJ of food consumed. Carcasses were dried in a convection oven at 100°C to constant weight (~72 h) and homogenized. The total fat content of the carcasses was obtained by extracting dried samples (~8 g) with light petroleum (40–60°C) in a Soxhlet extractor (Det-gras N, J.P. Selecta, Barcelona, Spain) and the dry defatted matters were used as a proxy of protein content. Livers were freeze-dried (FreeZone 6 Freeze Dryer, LABCONCO, Kansas City, MO, USA), and fat was extracted with light petroleum (40–60°C) using an ANKOM^{XT10} extractor (ANKOM Technology, Macedon, NY, USA). All determinations were carried out in duplicate.

Plasma analyses

Fasting plasma glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerides (TG), total phosphorus, albumin, plasma urea nitrogen, and C-reactive protein (CRP) were determined with the Vitros 350 Chemistry System (Ortho-Clinical Diagnostics, Raritan, NJ, USA). Low-density lipoprotein cholesterol (LDL-C) was computed from the values of TC, HDL-C, and TG using the Friedewald equation [31]. The plasma insulin concentration was determined with an enzyme immunoassay using the Rat/Mouse Insulin ELISA Kit (EZRMI-13K; EMD Millipore Corporation, Billerica, MA, USA).

Statistical analyses

The required number of rats (N = 9) was calculated using previously determined weight gain data (6 g/d standard deviation [SD] 0.95) and assuming a 25% difference in the mean, with a statistical power of 90% and a 5% significance level. Data are expressed as the mean ± SD of all values. A one-way analysis of variance (ANOVA) with Fisher's pair-wise comparison was performed using SPSS version 23.0 (IBM, Armonk, NY, USA).

Results

Energy intake, weight gain, and energy efficiency

Energy intake over the experimental period was significantly different ($P < 0.001$) among the groups. It was the lowest in the

non-supplemented gluten group (G; 0.30 MJ/d), with a slight but not significant increment observed with phosphorus supplementation (G+P; 0.33 MJ/d), whereas energy intake was significantly higher (~15%) in the lysine group (G+L; 0.36 MJ/d). However, rats supplemented with the combination lysine and phosphorus (G+L+P; 0.44 MJ/d) had a significantly higher (~45%) energy intake than the other groups (Fig. 1A). It is noteworthy that changes in energy intake were manifested by the introduction of the different diets and sustained throughout the experimental period (Supplementary Fig. 1).

Initial body weight did not differ among the different groups ($P = 0.998$), and this became statistically significant ($P < 0.001$) for final body weight by the end of the experimental period (Table 2).

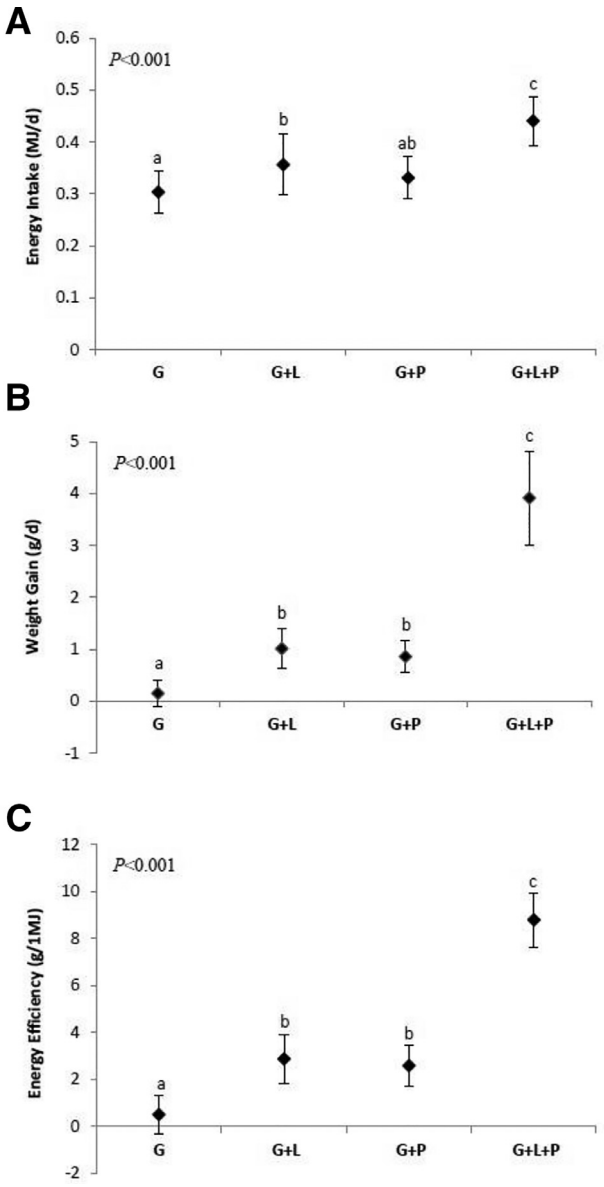

Fig. 1. Average daily energy intake (**A**), weight gain (**B**), and energy efficiency (**C**) of the four groups of rats over the 9-wk experimental period. Data are expressed as the mean ± SD of all values. A one-way analysis of variance analysis was performed. Values in the same row with different superscript letters were significantly different based on Fisher's pair-wise comparison. Significance was set at $P < 0.05$. G, gluten protein (10%w/w); G+L, gluten protein (10%w/w) and lysine (0.6%w/w); G+P, gluten protein (10%w/w) and phosphorus (0.3%w/w); G+L+P, gluten protein (10%w/w), lysine (0.6%w/w) and phosphorus (0.3%w/w).

Average weight gain of groups G+L and G+P did not differ at ~1 g/d and were significantly higher than that of group G (0.15 g/d) but lower than that of group G+L+P (3.9 g/d). Notably, weight gain of the G+L+P group was much higher than that of the other groups (Fig. 1B).

Energy efficiency among the experimental groups followed a similar pattern to that of weight gain. G+L (2.9 g/MJ) and G+P (2.6 g/MJ) groups had similar energy efficiencies, which were significantly higher (0.5 g/MJ) than that of group G. However, the energy efficiency of G+L+P (8.8 g/MJ) was highly potentiated and was significantly higher than that of the other groups (Fig. 1C).

Carcass and liver composition

Body composition analysis showed that body water (%) of group G+L+P was significantly lower ($P < 0.001$), whereas its body fat (%) was significantly higher ($P < 0.001$) than those of the other groups. Groups G, G+L, and G+P had similar body water (%) and fat (%). The protein status of the carcasses, approximated by the dry-defatted matters (%), of the P-containing diets (G+P and G+L+P) was significantly higher ($P = 0.005$) than that of the other groups (Table 2). In general, the observed ~10% decrease in body water (%) of G+L+P group was associated with a ~10% increase in fat (%; Table 2).

Liver weight was found to be significantly affected by the different diets ($P < 0.001$); it was increased by ~2 g in the G+L and G+P groups and almost doubled in the G+L+P group compared with the G group (Table 2). However, when the liver weight was expressed as g/100 g body weight, the differences among the groups became less pronounced, although significant difference remained ($P = 0.028$) between G and G+L+P. Liver water (%) was significantly different among the groups ($P = 0.021$), in which that of the phosphorus-containing diets (G+P and G+L+P) was lower than the others. In contrast, liver fat (%) of the phosphorus-containing diets was higher than that of the other groups ($P < 0.001$). Hepatic defatted matters (%) of G+P were significantly lower ($P = 0.003$) than those of the other groups.

Plasma metabolites

Plasma total phosphorus concentration of the phosphorus-containing diets (G+P and G+L+P) was significantly higher ($P < 0.001$) than that of the other groups. The plasma albumin concentration was significantly different ($P < 0.001$) between the groups, in which group G+P had the lowest level, followed by G, G+L, and G+L+P groups. Plasma urea nitrogen was significantly different among the groups ($P = 0.008$); G and G+L groups had similar levels that were significantly reduced in the phosphorus-containing groups, especially the G+P group. Plasma CRP concentration was similar among the four groups (Table 3).

Plasma glucose concentrations did not differ among the different groups, although that of G+L+P group was slightly higher. Plasma insulin concentration was significantly different among the groups ($P = 0.001$), mainly owing to its high level in the G+L+P group, and consequently, homoeostatic model assessment for insulin resistance (HOMA-IR) followed a similar pattern ($P = 0.007$). The lipid profile was affected by the dietary manipulation, except for that of TC. Phosphorus-containing groups (G+P and G+L+P) maintained similar LDL-C concentrations, which were lower than those of the other groups ($P = 0.039$), whereas the HDL-C of the G group was significantly higher ($P = 0.039$) than that of the other groups. Plasma TG concentrations were significantly different among groups ($P < 0.001$); the low plasma TG levels in G and G+P groups were increased by the addition of lysine (G+L) and further increased by the combination of lysine plus phosphorus (Table 3).

Table 2
Body and liver composition of rats maintained for 9 wk on a gluten diet supplemented with lysine or phosphorus

Parameter	G	G+L	G+P	G+L+P	*P*-value
Body weight and composition					
Initial body weight (g)	227.3 ± 17.5	225.9 ± 18.6	227.3 ± 18.2	226.9 ± 17.4	0.998
Final body weight (g)	236.9 ± 22.4[a]	289.7 ± 36.3[b]	281.4 ± 23.7[b]	473.6 ± 60.4[c]	<0.001
Water (%)	67.95 ± 1.17[a]	67.93 ± 0.74[a]	65.11 ± 1.49[a]	55.60 ± 6.75[b]	<0.001
Fat (%)	6.16 ± 0.43[a]	6.24 ± 0.79[a]	7.76 ± 1.22[a]	16.29 ± 5.78[b]	<0.001
Defatted matters (protein) (%)	25.89 ± 1.02[a]	25.83 ± 0.94[a]	27.13 ± 0.79[ab]	28.11 ± 2.61[b]	0.005
Liver weight					
Liver wet weight (g)	6.50 ± 0.66[a]	8.59 ± 1.34[b]	8.45 ± 1.57[b]	15.11 ± 2.95[c]	<0.001
Liver wet weight (g/100 g body weight)	2.75 ± 0.15[a]	2.96 ± 0.29[ab]	2.98 ± 0.34[ab]	3.18 ± 0.38[b]	0.028
Liver composition from the wet weight					
Liver water (%)	71.96 ± 1.03[a]	71.54 ± 1.25[a]	69.18 ± 2.94[b]	69.31 ± 3.44[b]	0.021
Liver fat in wet (%)	3.05 ± 0.83[a]	3.36 ± 0.90[a]	8.03 ± 4.25[b]	6.37 ± 2.33[b]	<0.001
Liver defatted matters (%)	25.00 ± 1.06[a]	25.10 ± 0.92[a]	22.79 ± 1.57[b]	24.32 ± 1.96[a]	0.003

G, gluten protein (10%w/w); G+L, gluten protein (10%w/w) and lysine (0.6%w/w); G+P, gluten protein (10%w/w) and phosphorus (0.3%w/w); G+L+P, gluten protein (10%w/w), lysine (0.6%w/w) and phosphorus (0.3%w/w).
Data are expressed as the mean ± SD of all values.
A one-way analysis of variance analysis was performed.
Values in the same row with different superscripts are significantly different based on Fisher's pair-wise comparison.
Significance was set at a $P < 0.05$.

Discussion

The present data demonstrated that growth measures, including energy intake, body weight, and composition, were variably affected by the addition of lysine or phosphorus or a combination of both to the diet. Energy intake improved from the time of lysine with or without phosphorus introduction to the diet in a manner that was sustained over the experimental period. Short-term regulation of food intake was hypothesized to be controlled by homeostasis, unlike that of long-term control believed to be regulated by nutrient requirements for growth [32]. In other words, optimal food intake is attained when absorbed nutrients are metabolized or cleared from the circulation; whereas when nutrients are not metabolized, food intake decreases to avoid accumulation and toxicities. In support, animals (rats and birds) were shown to select a proportion from different dietary sources in order to meet their phosphorus nutritional requirements [33,34], a mechanism known as phosphorus appetite [35]. In addition, under conditions of low lysine availability, rats become keen on selecting a lysine-containing diet, if available, to meet their needs for growth; otherwise, they decrease their food intake [36] to avoid consuming an amino acid-imbalanced diet (e.g., a gluten diet) [37]. The mechanism(s) behind the anorectic effect of low-lysine diets (gluten) is thought to be the consequence of a decline in the concentration of lysine in the brain [38,39] or an adaptation to protect against the potential toxicity of amino acid accumulation resulting from reduced protein synthesis [40].

In a nutshell, the results presented here imply that the acute improvement in energy intake was likely to have been mediated via the effects of lysine, phosphorus, or a combination of the two on homeostasis or postprandial metabolism. The ability of low-protein diets to alter energy intake [14,41] may implicate protein metabolism in this process. The modest effects of lysine or phosphorus addition to diet on energy intake (~15%) is in line with previous observations, in which energy consumption was reported to be enhanced by the addition of lysine [14,42] or phosphorus [26] to the diet. The amplified increase (~45%) in energy intake by the simultaneous addition of lysine and phosphorus was inversely associated with plasma urea nitrogen, a product of protein breakdown [36], thereby indicating the involvement of protein metabolism in energy intake regulation.

The resulting blunted growth by gluten diet consumption was in line with others [14,41,43], thereby lending further support to the failure of incomplete proteins to support growth, body weight, and protein synthesis [10,11,44]. Therefore, cereal-based diets are likely to compromise growth and maintenance. Furthermore, the observed modest growth improvement by lysine addition, as compared with other studies [9,11,12,15,45], may be explained by the use of phosphorus-free mineral mix, especially because weight gain is known to be dependent on phosphorus availability [25,26]. However, the prominent weight gain on diets containing both lysine and phosphorus suggests that the improvement in the amino acid profile alone is not sufficient enough to support growth. Weight gain improvements of the supplemented diets were related to both energy intake and efficiency [32] with the later exerting a more predominant effect, as indicated by its high magnitude of increase. As a result, the pattern of variations in weight gain was found to parallel that of energy efficiency. The increase in weight gain was not associated with a proportional increase in body composition, in which water (%) of the combination (G+L+P) was lower than that of the other groups. This difference is likely to have been attributed to the pronounced increase in fat (%) of this group.

In the phosphorus-containing diets, the reduction in plasma urea nitrogen was associated with an increase in body protein (%), thus implying that protein metabolism was highly affected by phosphorus rather than by lysine availability. Furthermore, the ability of phosphorus to lower plasma urea nitrogen (improvements in protein metabolism) was evident even under the condition of incomplete protein or reduced energy intake. Similarly, the effect of phosphorus on hepatic fat (%) was not likely to be related to energy intake or protein quality. Moreover, the ability of lysine but not phosphorus consumption to improve serum albumin levels was in line with other studies [12,15,46] and this may be due to fact that lysine is a major component of albumin [47].

Looking at the lipid profile, plasma TG levels were highly correlated with that of insulin ($r = 0.523$; $P = 0.001$) and HOMA-IR ($r = 0.535$; $P = 0.000$), and this may imply that the reported increase in TG levels, especially in the combination (G+P) group, may have been attributed to changes in insulin status, which is known to increase plasma TG [48]. Increased levels of insulin, glucose, and TG in the G+L+P group may have been attributed to their elevated fat content (more than double that of the other groups). Adipose

Table 3
Plasma metabolites of rats maintained for 9 wk on a gluten diet supplemented with lysine, phosphorus, or a combination of both

Parameter	G	G+L	G+P	G+L+P	*P*-value
Total phosphorus (mg/dL)	5.99 ± 1.36^{a}	5.79 ± 1.12^{a}	7.49 ± 0.78^{b}	8.26 ± 0.64^{b}	<0.001
Albumin (g/dL)	3.13 ± 0.16^{a}	3.41 ± 0.21^{b}	2.77 ± 0.22^{c}	3.74 ± 0.32^{d}	<0.001
Plasma urea nitrogen (mg/dL)	15.67 ± 4.21^{ab}	16.00 ± 5.40^{a}	9.30 ± 5.77^{c}	11.70 ± 2.98^{bc}	0.008
CRP (mg/L)	0.89 ± 1.36	0.60 ± 0.70	1.30 ± 1.57	0.70 ± 0.48	0.519
Glucose (mg/dL)	177 ± 60.3	184.7 ± 63.2	171 ± 56.4	233.5 ± 42.2	0.07
Insulin (μg/mL)	491 ± 58.7^{a}	516 ± 52^{a}	521.2 ± 177.4^{a}	703.4 ± 117.4^{b}	0.001
HOMA-IR	6.35 ± 2.61^{a}	6.91 ± 2.83^{a}	6.86 ± 5.29^{a}	11.60 ± 2.50^{b}	0.007
Total-cholesterol (mg/dL)	89.22 ± 13.80	84.70 ± 13.27	75.20 ± 10.13	81.20 ± 6.78	0.065
LDL-cholesterol (mg/dL)	16.53 ± 4.32^{ab}	17.50 ± 5.29^{a}	12.32 ± 5.41^{b}	12.32 ± 4.37^{b}	0.039
HDL-cholesterol (mg/dL)	67.33 ± 10.30^{a}	59.50 ± 9.57^{b}	58.10 ± 6.31^{b}	57.20 ± 4.92^{b}	0.039
TG (mg/dL)	26.78 ± 6.48^{ab}	38.50 ± 9.55^{a}	23.90 ± 5.43^{b}	58.40 ± 26.43^{c}	<0.001

CRP, C-reactive protein; G, gluten protein (10%w/w); G+L, gluten protein (10%w/w) and lysine (0.6%w/w); G+P, gluten protein (10%w/w) and phosphorus (0.3%w/w); G+L+P, gluten protein (10%w/w), lysine (0.6%w/w) and phosphorus (0.3%w/w); HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; TG, triacylglycerides.
Data are expressed as the mean ± SD of all values.
A one-way analysis of variance analysis was performed.
Values in the same row with different superscript letters were significantly different based on Fisher's pair-wise comparison. Significance was set at $P < 0.05$.

tissue content is known to be paralleled by an increase circulating free fatty acid that consequently favors the development of IR, which in turn increases TG hepatic synthesis by inhibiting lipoprotein lipase [48]. The reduced plasma LDL-C, an $ApoB_{100}$ containing lipoprotein, levels in the phosphorus-containing groups may in part relate to the ability of phosphorus to suppress postprandial $ApoB_{100}$ [21]. Overall, the relation between plasma phosphorus and the lipid profile is controversial, as a high serum phosphate level was linked with both an increase and decrease in TC and LDL-C, whereas TG levels were mainly inversely related [49,50]. On the other hand, the observed strong correlation between energy efficiency, TG ($r = 0.68$; $P = 0.000$) and insulin ($r = 0.596$; $P = 0.000$) levels may implicate energy efficiency in this process. Under low-energy efficiency conditions, substrates are mainly geared toward oxidation (catabolic) rather than storage or synthesis (anabolic). A condition that ultimately inhibits the production of both insulin and TG.

In general, weight measures were highly enhanced by the combination of lysine and phosphorus, thereby indicating that phosphorus, in addition to improving the amino acid profile, is required to support growth [26]. To this end, a substantial improvement in nitrogen retention was reported in young men after the ingestion of a lysine-fortified wheat gluten diet when supplemented with micronutrients, including phosphorus [46], whereas the increase in lysine ingestion alone failed to do so [16,25]. This supports our hypothesis that concomitant improvement in amino acid profile and phosphorus availabilities is required to enhance growth.

Conclusion

A combination of both lysine and phosphorus is needed to potentiate growth when wheat gluten is the sole source of protein in the diet [9,12]. Current findings support our findings that the varied effects of lysine supplementation on growth between animal and human interventions [9,12,15,16] are related to phosphorus bioavailability. In addition, phosphorus was found to have an independent positive effect on measures of protein metabolism (e.g., body protein [%], plasma urea nitrogen). Thus, human interventions should address both the improvement in amino acid profile and phosphorus availability to better optimize outcomes.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2018.11.009.

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