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Acute and Chronic Effects of Honey and Its Carbohydrate Constituents on Calcium Absorption in Rats

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The effects of honey and its carbohydrate constituents (glucose, fructose, and raffinose) on calcium absorption in rats were investigated in acute and chronic feeding studies. In the acute study, rats (n = 120) were gavaged with an oral solution consisting of (a) 10 μCi ^{45}Ca, (b) 25 mg of calcium as calcium acetate, and (c) one of the following: 0 mg of honey (control), or 200, 500, or 800 mg of honey, a glucose−fructose mixture, 10.75 mg of raffinose, or 200 mg of raffinose. Another group received ^{45}Ca intraperitoneally. Femurs were collected 2 days later and analyzed for ^{45}Ca content. Rats given 500 and 800 mg of honey showed 25.5 and 33.6% increases in calcium absorption (P < 0.05), respectively, over the control group. Groups given the glucose−fructose mixture or 200 mg of raffinose had a significantly higher increase in calcium absorption than the control group (17.1 and 25.6%, respectively). In the chronic study, rats (n = 96) were fed for 8 weeks with either 0% honey (control), 5% honey, 10% honey, or a glucose−fructose−raffinose (GFR) mixture. Femurs of GFR-fed rats had significantly lower calcium content, ^{45}Ca absorption, width, and BMD (at distal region) than control rats. Groups fed honey did not show the negative effects of GFR on bone, but had no advantage over the control group. No significant differences were observed in femur length, density, strength, or BMC among any treatment group compared to the control group. These results indicate that although a positive dose–response effect of honey and its carbohydrate constituents on calcium absorption was observed in the acute study, this effect disappeared upon long-term feeding in rats, implying adaptation had occurred.

KEYWORDS: Honey; nondigestible carbohydrates; calcium absorption; ^{45}Ca; rats

INTRODUCTION

The ingestion of certain nondigestible carbohydrates has been reported to increase calcium absorption in rats (*1–6*) and humans (*7–9*). Although the exact mechanisms contributing to this event have not been clearly elucidated, several have been proposed. In the gastrointestinal (GI) tract, nondigestible carbohydrates resist hydrolysis by digestive enzymes, pass through the small intestine, and eventually reach the cecum and colon. At these sites, they are fermented by the residing bacteria to produce byproducts such as short-chain fatty acids (SCFA), resulting in a decrease in intestinal pH (*10, 11*), which leads to an increase in mineral solubility (such as calcium) (*11–13*). Furthermore, in vitro studies based on human intestinal Caco-2 cells suggested that nondigestible carbohydrates indirectly open tight junctions in the epithelial cells by increasing intracellular calcium ion concentration. The induction of this intracellular calcium signaling results in the activation of paracellular transport of calcium (*14, 15*). It has also been postulated that nondigestible carbohydrates increase active calcium transport by the activation of calbindin-D9k (*16, 17*). A combination of these events has been associated with increases in net intestinal calcium absorption.

Previous studies investigating the effects of nondigestible carbohydrates on mineral absorption are based on the administration of the purified forms of these nondigestible carbohydrates (*1–5, 15, 18*). Foods containing nondigestible carbohydrates such as honey may have similar calcium uptake-enhancing properties, but matrix effects are less well-known. Honey is a commonly used sweetener consisting of the carbohydrates fructose, glucose, and raffinose. Fructose and glucose are the dominant carbohydrates in honey (30.9−44.3 and 22.9−40.8% by weight, respectively) (*19, 20*). One of the few studies investigating the effects of honey on calcium metabolism reported a higher calcium retention in infants fed honey-supplemented milk than control (*21*). Various studies based on animal models have also observed the calcium uptake-enhancing effects of raffinose (*1, 4, 5*), which is present in honey (*22*).

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In the present study, we investigated the acute and chronic effects of honey and its carbohydrate constituents (specifically glucose, fructose, and raffinose) on calcium absorption in growing rats by applying an isotopic tracer method (based on femur uptake of ^{45}Ca). This method allows direct quantification of mineral absorption in relation to the amount consumed, thus making it a more sensitive method than the metabolic balance technique used in previous studies (*21, 23, 24*). Furthermore, we investigated the calcium absorption effects of the nondigestible carbohydrates (e.g., raffinose) as individual components and as part of the food matrix (i.e., in the form of honey).

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Harlan Inc., Indianapolis, IN; 6 and 8 weeks old) were housed in individual stainless steel cages maintained in an environmentally controlled room (22–24 °C, 40–60% humidity) with a 12 h light/dark cycle. Rats were given free access to deionized water and respective treatment diets. All protocols were approved by the Institutional Animal Care and Use Committee at Purdue University, West Lafayette, IN.

Materials. Liquid honey (Pure U.S. Fancy White Honey, Honey Acres, Ashippun, WI) was used. The moisture content of honey was determined by measuring the refractive index at 20 °C. The average refractive index values were converted to honey moisture content using the table listed in AOAC method 969.38 (*25*). The carbohydrate constituents of honey (glucose, fructose, raffinose) were determined by high-performance liquid chromatography (HPLC) using AOAC method 977.20 (*25*). By analysis, this honey contained 16.1% moisture, 33% glucose, 37% fructose, and 1.16% raffinose.

Experimental Design. *Acute Study.* After 1 week of adaptation on an AIN-93G diet (Dyets Inc., Bethlehem, PA) (*26*), 8-week-old rats were randomly divided into eight groups ($n = 15$/group). Seven groups of rats were gavaged with an oral solution of 10 μCi of ^{45}Ca, 25 mg of calcium acetate, and their assigned treatment of one of the following: 0 mg (control), 200, 500, or 800 mg honey (on dry weight basis), glucose−fructose mixture, 10.75 mg of raffinose (low raffinose), or 200 mg of raffinose (high raffinose). Test solutions made of the glucose−fructose mixture and low raffinose were formulated on the basis of the amounts of these carbohydrates in 800 mg of honey on a dry weight basis. To mimic 100% calcium absorption, the eighth group of rats was injected intraperitoneally (ip group) with 5 μCi of ^{45}Ca and gavaged with an oral solution of 25 mg of calcium as calcium acetate. Rats were fasted for 4 h prior to gavage and for 2 h after gavage prior to being fed the AIN-93G diet ad libitum. Two days after the gavage or ip injection, rats were sacrificed and had their right femurs harvested for ^{45}Ca analysis.

Chronic Study. Six-week-old rats were acclimatized to laboratory conditions for 1 day and then were randomly divided into four groups ($n = 24$/group): (i) control, (ii) 5% honey, (iii) 10% honey, and (iv) glucose−fructose−raffinose (GFR) mixture. The AIN-93G diet was used as the control diet. The 10 and 5% powdered honey diets were made by Dyets, Inc. The GFR diet was made in our laboratory and was formulated on the basis of the AIN-93G diet with proportional substitution of cornstarch according to the amounts of glucose, fructose, and raffinose in 10% honey. These diets were isocaloric with the caloric density maintained at 3.8 kcal/g. The diet composition is listed in **Table 1**.

After 8 weeks of intervention, 14 rats from each group were gavaged with an oral solution consisting of a mixture of 10 μCi of ^{45}Ca, 25 mg of calcium as calcium acetate and honey or GFR mixture on a dry weight basis equivalent to 5g powdered diet. The remaining 10 rats from each group were gavaged with an oral solution consisting of a mixture of 25 mg of calcium as calcium acetate and one of the treatments above and then injected with 5 μCi of ^{45}Ca intraperitoneally (ip group). Rats were sacrificed 2 days after gavage and had both femurs harvested. Femurs were wrapped in saline-soaked gauze and stored at 4 °C until evaluated for bone parameters.

Bone Analyses. *Acute Study.* Femurs were dissolved overnight in 3 mL of concentrated HNO_3 and diluted to 25 mL using 1 N HCl containing 0.5% La as $LaCl_3$. From this, a 1 mL aliquot was combined with 15 mL of liquid scintillation cocktail (Ecolite, MP Biomedicals, Irvine, CA) and counted for ^{45}Ca radioactivity (LS6500; Beckman Coulter Inc., Fullerton, CA). Percent calcium absorption was calculated by using the following formula:

$$\text{calcium absorption (\%)} = (\%\ ^{45}\text{Ca dose in femur of oral group}/\%\ ^{45}\text{Ca dose in femur of ip group}) \times 100\%$$

Table 1. Composition (Grams per Kilogram of Dry Diet) of the Isocaloric Experimental Diets Fed to the Rats during the Chronic Study (Caloric Density = 3.8 kcal/g)

ingredient	AIN-93G	5% honey[a]	10% honey[a]	GFR[b]
honey	0	59.59	119.19	0
glucose	0	0	0	39.33
fructose	0	0	0	44.10
raffinose	0	0	0	1.38
sucrose	100	100	100	100
cornstarch	397.49	347.49	297.49	303.9
casein	200	200	200	200
L-cystine	3	3	3	3
Dyetrose	132[c]	132	132	132
soybean oil with TBHQ	70	70	70	70
cellulose	50	50	50	50
mineral mix	35	35	35	35
vitamin mix	10	10	10	10
choline bitartrate	2.5	2.5	2.5	2.5
calcium[d]	4.88	4.98	4.74	4.86

[a] Based on dry weight basis. [b] GFR, diet consisting of glucose−fructose−raffinose mixture. [c] Dextrin was used in AIN93G instead of Dyetrose. [d] Determined chemically using atomic absorption spectrometry.

Chronic Study. Right femurs from the chronic study were scanned by dual X-ray absorptiometry (DEXA; DXP-IQ, GE/Lunar, Madison, WI). Bone mineral density (BMD) and bone mineral content (BMC) of the proximal, midshaft, and distal regions were assessed, which were defined as 25, 50, and 75% of the whole femur length, respectively. Following scanning, femurs were dissolved overnight in 3 mL of concentrated HNO_3 and diluted to 25 mL using 1 N HCl containing 0.5% La as $LaCl_3$. From this, appropriate dilution of each sample was prepared and total calcium in the femur was assayed using atomic absorption spectrometry (A Analyst 300; Perkin-Elmer, Inc., Norwalk, CT). Amount of calcium per gram of femur was calculated by dividing the amount of calcium in the femur by the weight of the femur. Percent calcium absorption based on the femur uptake of ^{45}Ca was also assessed using the method and formula outlined in the acute study section.

Left femurs from the chronic study were measured for length and midshaft width using calipers. They were also measured for density by underwater weighing method. Following these measurements, femurs were then analyzed for strength on the basis of a three-point bending test performed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Data were expressed as peak force (kilograms) necessary to fracture the femur at midshaft using a test speed of 1 mm/s.

Statistical Analysis. Treatment means were analyzed using the SAS statistical program (version 9.1; SAS Institute, Cary, NC) by one-way ANOVA, and differences between treatments were grouped by Duncan's test. Data are expressed as means ± SD. Differences were considered to be significant when $P < 0.05$.

RESULTS

Acute Study. A dose–response effect of honey on calcium absorption was observed (**Figure 1**). Femurs of rats gavaged with 500 and 800 mg of honey had significantly higher calcium absorption efficiency than the control group and than rats administered 200 mg of honey ($P < 0.05$). The highest dose of honey (800 mg) produced a 33.6% increase in calcium absorption, followed by 500 mg of honey, which produced a 25.5% increase. The carbohydrate constituents in honey also had an

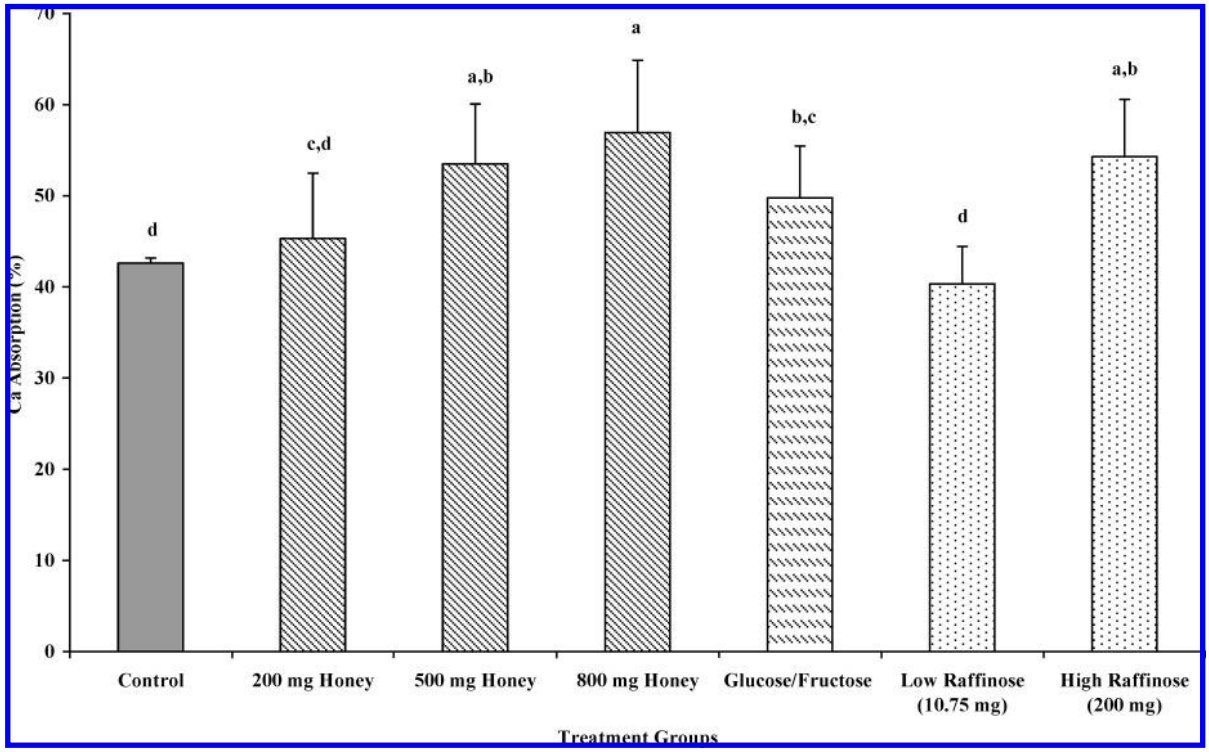

Figure 1. Comparison of calcium absorption (percent) of rats fed different concentrations of honey and its carbohydrate constituents in the acute study. Values were calculated on the basis of femur uptake of ^{45}Ca. Treatment groups with different letters indicate significant differences between groups ($P < 0.05$). Values are means ± SD.

Table 2. Effects of Honey and Its Carbohydrate Constituents on Body Weight and Feed Intake of Rats Administered the Respective Treatments for 8 Weeks[a]

variable	control	5% honey[b]	10% honey[b]	GFR[c]
initial body wt (g)	175.83 ± 5.90a	175.21 ± 10.22a	174.83 ± 9.06a	174.54 ± 4.81a
final body wt (g)	391.21 ± 24.29a	379.08 ± 25.84a	380.75 ± 19.31a	388.42 ± 25.02a
av wt gain (g)	215.38 ± 23.37a	203.88 ± 24.29a	205.92 ± 16.38a	213.88 ± 26.02a
total food intake (g)	1159.33 ± 78.62b	1307.88 ± 115.47a	1251.42 ± 113.73a	1162.75 ± 139.21b
total calcium intake (g)	5636.50 ± 390.06b	6505.17 ± 564.39a	5889.73 ± 567.96b	5649.07 ± 662.40b
feed efficiency (g of wt gain/g of diet)	0.186 ± 0.021a	0.156 ± 0.015b	0.166 ± 0.021b	0.186 ± 0.027a

[a] Values are means ± SD. Values with different letters in a row are significantly different ($P < 0.05$). [b] Based on dry weight basis [c] GFR, diet consisting of glucose−fructose−raffinose mixture.

effect on calcium absorption. Groups given the glucose−fructose mixture or 200 mg of raffinose had a significantly higher increase ($P < 0.05$) in calcium absorption than control (17.1 and 25.6%, respectively). However, calcium absorption efficiency of rats administered the low dose of raffinose (10.75 mg) did not significantly differ from control ($P = 0.21$).

Chronic Study. *Body Weight and Feed Intake.* Total food intake was significantly higher in the 5 and 10% honey groups than control and GFR groups ($P < 0.05$). However, all groups had similar final body weights and average weight gains at the end of the 8 week feeding period ($P = 0.23$ and 0.22, respectively). Thus, rats fed the honey diets had significantly lower food efficiency ratios (grams of weight gain per gram of diet) than rats fed control and GFR diets ($P < 0.05$) (**Table 2**).

Calcium Absorption and Femur Measurements. Rats fed the GFR diet had significantly ($P < 0.05$) lower amounts of calcium per gram of femur than control and honey-fed groups and lower calcium absorption efficiency (in terms of femur uptake of ^{45}Ca) than control and 5% honey-fed groups (**Table 3**). These parameters, however, did not differ significantly among the two honey and control groups. In addition, femur width was significantly lower in the 5% honey and GFR groups compared to the control group ($P = 0.02$), but was not different for rats given 10% honey. Other femur measures, that is, length ($P = 0.55$), density ($P = 0.37$), strength (in terms of peak force to breaking) ($P = 0.17$), femur BMC [at proximal ($P = 0.12$), midshaft ($P = 0.11$), and distal ($P = 0.88$) regions], or femur BMD [at proximal ($P = 0.63$) and midshaft ($P = 0.19$) regions] did not show any significant differences among the treatment groups (**Table 3**). However, femur BMD at the distal region was significantly lower in the GFR group than control group ($P = 0.02$) (**Table 3**).

DISCUSSION

In the acute feeding study, honey and its carbohydrate constituents (glucose, fructose, and raffinose) produced a dose–response effect on calcium absorption. Various concentrations of honey (500 and 800 mg) and its carbohydrate constituents (a mixture of glucose−fructose, 10.75 mg of raffinose, or 200 mg of raffinose) increased calcium absorption by 17.0−33.6% over the control. Interestingly, rats fed with 800 mg of honey had a significantly higher percent calcium absorption than those fed the glucose−fructose mixture or a low dose of raffinose, even though the latter diets were

Table 3. Effects of Honey and Its Carbohydrate Constituents on Femur Measurements in Rats Administered the Respective Treatment Diets for 8 Weeks[a]

variable	control	5% honey[b]	10% honey[b]	GFR[c]
femur length (mm)	37.1 ± 0.64a	36.87 ± 0.83a	37.01 ± 0.78a	37.12 ± 0.88a
femur width (mm)	4.316 ± 0.225a	4.160 ± 0.220b	4.265 ± 0.189ab	4.163 ± 0.188b
femur density (g/cm^3)	1.453 ± 0.021a	1.465 ± 0.018a	1.457 ± 0.022a	1.460 ± 0.028a
femur strength (kg)	15.58 ± 2.72a	15.01 ± 2.23a	14.03 ± 2.82a	15.74 ± 3.19a
femur BMD[d] (g/cm^2)				
proximal[e]	0.229 ± 0.011a	0.228 ± 0.008a	0.226 ± 0.008a	0.226 ± 0.012a
midshaft[e]	0.227 ± 0.008a	0.225 ± 0.009a	0.221 ± 0.011a	0.226 ± 0.010a
distal[e]	0.216 ± 0.008a	0.211 ± 0.008ab	0.214 ± 0.009a	0.209 ± 0.010b
femur BMC[f] (g)				
proximal[e]	0.092 ± 0.008a	0.086 ± 0.009a	0.087 ± 0.010a	0.090 ± 0.010a
midshaft[e]	0.189 ± 0.011a	0.189 ± 0.015a	0.183 ± 0.011a	0.192 ± 0.014a
distal[e]	0.104 ± 0.009a	0.102 ± 0.009a	0.102 ± 0.007a	0.102 ± 0.009a
calcium content per g of femur (mg/g)	125.19 ± 6.24a	123.63 ± 3.89a	122.65 ± 7.04a	119.38 ± 5.58b
calcium absorption (%)[g]	38.99 ± 6.21a	38.28 ± 5.64a	37.21 ± 5.82ab	33.59 ± 4.27b

[a] Values are means ± SD. Values with different letters in a row are significantly different ($P < 0.05$). [b] Based on dry weight basis. [c] Diet consisting of glucose−fructose−raffinose mixture. [d] Bone mineral density. [e] Proximal, midshaft, and distal regions correspond to 25, 50, and 75% of the whole length of femur, respectively. [f] Bone mineral content. [g] Values were calculated based on femur uptake of ^{45}Ca.

formulated on the basis of the amounts of these carbohydrates in 800 mg of honey on a dry weight basis. This suggests that there may be other factors in honey that contribute to its calcium absorption enhancing effect. A dose of 500 mg of honey with 25 mg of calcium given as calcium acetate as fed to the rats during the acute study is equivalent to approximately 6 g of honey (on dry weight basis) or 1 teaspoon consumed with a cup of milk (containing an equivalent to 300 mg of calcium) in humans. This dose would be a practical and achievable dietary intervention to increase calcium absorption if effective.

The calcium absorption enhancing effect of honey observed in the acute study diminished with chronic feeding. Calcium absorption efficiency, total calcium content in femur, and other femur measures were not significantly improved by the presence of honey compared to the control, in contrast to observations in the acute study. Our observation is consistent with many other previous studies indicating that early nutritional benefits may not translate to long-term effects. When fed as a single meal, a diet containing 5% lactulose enhanced calcium absorption by 20%; however, this effect disappeared after chronic feeding (*1*). Similarly, acute feeding of whey protein concentrate was shown to increase calcium absorption by 18−36%; however, this calcium absorption enhancing effect was not observed with chronic feeding (*27*). Repeated balance studies in mineral retention experiments also showed that the stimulating effect of oligofructose on mineral retention was measurable in the first and second weeks of the experiment, but lost significance after 2 or 3 weeks (*11*).

The lack of long-term nutritional benefit may be attributed to several factors. Upon prolonged administration of a highly bioavailable calcium load, colonic calcium absorption adapts to maintain homeostasis. The parathyroid hormone−vitamin D mechanism may be suppressed by the initial increase in calcium absorption. Subsequently, this event down-regulates the active transport of calcium absorption (*1*). Besides physiological adaptation, mineral bioavailability may also be influenced by the age of the animal. Previous studies noted that younger animals showed higher mineral bioavailability than older rats. A study conducted by Pérez-Conesa et al. (*28*) observed a significant decrease in calcium and magnesium absorption in rats during an 18 day time lapse between the first and third mineral balance periods. Similarly, Coudray et al. (*6*) also reported that the effect of inulin in enhancing intestinal absorption of calcium and magnesium is significantly affected by the age of the rats. Younger rats have higher expression of calcium binding proteins (e.g., calbindin-D9k) in the intestines, which is positively associated with calcium absorption efficiency (*29*). Physiological changes due to aging may have blunted the overall calcium absorption enhancing effect of honey and its carbohydrates at the end of the chronic feeding study. The amount of calcium in the diet may also influence the magnitude of the effect of carbohydrates on calcium absorption. Previous studies that have demonstrated the calcium absorption enhancing property of nondigestible carbohydrates became more prominent when the diet contained higher than 5 g of calcium/kg of diet (*1, 11, 30*).

The negative effect of the carbohydrate constituents of honey (glucose, fructose, and raffinose) on calcium and bone parameters in the chronic feeding study was unexpected, given their enhancing effect on calcium absorption in the acute study. As with the acute study, calcium absorption was higher in the presence of honey than its carbohydrate constituents, and this benefit was extended to some bone parameters in the chronic study. Differences in calcium absorption among rats fed the control diet, diet with honey, and diet with added carbohydrates may be attributed to food matrix effects. Werner et al. (*31*) observed that less calcium was absorbed from a test meal than from a pharmaceutical calcium preparation, even though they contained similar amounts of calcium. However, studies investigating food matrix effects on mineral absorption have shown mixed results. Several controlled human feeding studies indicated no significant differences in the fractional absorption of total calcium among milk-fortified foods and calcium salts (*32–34*). Soy milk fortified with tricalcium phosphate reduced calcium absorption compared to milk (*35*), whereas a marginal increase in calcium absorption was observed when cow's milk was fortified with a milk-derived calcium salt (*36*). In another study, a positive effect in calcium absorption was observed from the consumption of bread fortified with calcium sulfate (*37*). The supplementation of bread and dairy products with caseinophopshopeptides did not increase calcium absorption (*38, 39*), but supplementation at the same level in rice-based cereals resulted in improved calcium absorption (*40*). These results highlight the influence of the food matrix on calcium bioavailability.

Various studies have shown that short-term feeding of nondigestible carbohydrates produced a positive effect on calcium absorption in humans. Adolescents given 15 g of oligofructose three times daily for 9 days were observed to have significantly higher fractional calcium absorption than when fed sucrose as a control treatment (*7*). In another study by the same research group, a dose–response effect of lactulose (5−10 g/day) on calcium absorption was observed in postmenopausal women

after 9 days of consumption (*41*). This group further demonstrated a similar effect after a 9 day administration of transgalactooligosaccharides (TOS; 20 g/day) (*8*). Although the exact mechanisms accounting for the influence of these nondigestible carbohydrates remain undefined, TOS may have directly increased calcium uptake by the bones and/or inhibited bone resorption because the increase in calcium absorption was not accompanied by an increase in urinary calcium excretion (*8*). These observations and our data indicate that an intermittent consumption of honey may be a better approach for increasing calcium absorption than continuous feeding. For acute or intermittent feeding, coating a food typically consumed with a calcium source (e.g., sweetened cereal−milk combination) with honey may be of more health benefit than coating the cereal with carbohydrate constituents of honey (e.g., glucose, fructose, and/or raffinose).

In conclusion, acute feeding of honey and its carbohydrate constituents (glucose, fructose, and raffinose) enhanced calcium absorption that translates to skeletal benefits. However, this calcium absorption enhancing effect did not persist with chronic long-term feeding in growing rats. Further studies are needed to explore ways to prolong the calcium absorption enhancing effect of honey and its carbohydrate constituents, possibly through intermittent feeding or other approaches.

ABBREVIATIONS USED

GI, gastrointestinal; SCFA, short-chain fatty acids; HPLC, high-performance liquid chromatography; GFR, glucose−fructose−raffinose; ip, intraperitoneal; DEXA, dual X-ray absorptiometry; BMD, bone mineral density; BMC, bone mineral content; TOS, *trans*-galactooligosaccharides.

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