J Pediatr Endocr Met 2011;24(3-4):147–153 © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/JPEM.2011.009

A nutritional intervention study with hydrolyzed collagen in pre-pubertal Spanish children: influence on bone modeling biomarkers

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Abstract

Aim: The aim of the study was to investigate the influence of dietary intake of commercial hydrolyzed collagen (Gelatine Royal®) on bone remodeling in pre-pubertal children.
Methods: A randomized double-blind study was carried out in 60 children (9.42±1.31 years) divided into three groups according to the amount of partially hydrolyzed collagen taken daily for 4 months: placebo (G-I, n=18), collagen (G-II, n=20) and collagen+calcium (G-III, n=22) groups. Analyses of the following biochemical markers were carried out: total and bone alkaline phosphatase (tALP and bALP), osteocalcin, tartrate-resistant acid phosphatase (TRAP), type I collagen carboxy-terminal telopeptide, lipids, calcium, 25-hydroxyvitamin D, insulin-like growth factor-1 (IGF-1), thyroid-stimulating hormone, free thyroxin and intact parathormone.
Results: There was a significantly greater increase in serum IGF-1 in G-III than in G-II ($p<0.01$) or G-I ($p<0.05$) during the study period, and a significantly greater increase in plasma tALP in G-III than in G-I ($p<0.05$). Serum bALP behavior significantly ($p<0.05$) differed between G-II (increase) and G-I (decrease). Plasma TRAP behavior significantly differed between G-II and G-I ($p<0.01$) and between G-III and G-II ($p<0.05$).
Conclusion: Daily dietary intake of hydrolyzed collagen seems to have a potential role in enhancing bone remodeling at key stages of growth and development.

Keywords: bone; children; protein.

Introduction

From the age of 5 to 15 years, children establish important metabolic bases that determine their health as adults (1, 2). Nutrition during this period is one of the main factors for the avoidance of long-term diseases such as osteoporosis, arthritis, obesity and cardiovascular disease (3). There is an increasing worldwide prevalence of osteoporosis, which is characterized by a low bone mass and changes in bone microarchitecture that predispose to fractures (4). Although maximum bone mass has a major genetic component, it is influenced by nutrition and physical activity during early stages of life. Bone remodeling depends on the activity of osteoblasts (bone-forming cells) and osteoclasts (bone-destroying cells). Bone comprises an inorganic mineral compound, hydroxyapatite (calcium and phosphorus), and an organic matrix that is 90% type I collagen with a non-collagen component that contains different proteins such as osteocalcin, osteonectin, bone sialoprotein and proteoglycans. Before bone formation ceases in late adolescence, the intake of calcium, phosphorus and quality protein is of great importance in this process (5–7). Studies have shown a positive association between protein intake and bone mineral status in children and adolescents (7, 8), young women aged 18–31 years (9) and the elderly (10–12). In fact, collagen, a major protein component of bone, has been introduced for the treatment of osteoporosis and arthritis with satisfactory results, reducing resorption biomarkers and increasing formation biomarkers (13–15). Collagen represents a family of macromolecules with common structural characteristics that are synthesized by fibroblasts from pro-collagen. Collagen plays an important role in organ structures (e.g., skin, tendons, cartilage) and bone matrix-cell interactions during development (16). The objective of the present study was to investigate possible effects of the nutritional intake of hydrolyzed collagen in childhood on bone modeling markers.

Experimental methods

Subjects

A total of 97 healthy children between 6 and 11 years of age were recruited at three primary schools in Granada (Spain). Inclusion

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criteria were absence of disease, pre-pubescence (Tanner score I or II), signing of informed consent by parents and expectation of completing the study. Exclusion criteria were bone disease, metabolic disease, hepatic, renal or thyroid malfunction, intake of drugs that could affect bone metabolism and fluctuating dietary patterns. After application of the criteria, the final sample comprised 71 children. The study was conducted according to the Helsinki Declaration (2004), EEC Good Clinical Practice recommendations (document 111/3976/88, July 1990) and current Spanish legislation regulating clinical research on humans (Royal Decree 561/1993 on clinical trials), and the protocol was approved by the ethics committee of San Cecilio University Hospital (Granada, Spain).

Study design

The 71 children were randomly assigned to one of three groups for administration of placebo (G-I, n=24), partially hydrolyzed collagen (gelatine) (G-II, n=21) or gelatine supplemented with 0.935 mg of gluconate and lactate calcium in 100 g of collagen (G-III, n=26). All gelatine products were supplied by Royal® (Kraft Foods Europe, Barcelona, Spain), who prepared the placebo product specifically for this study.

The children consumed 250 mL of the product daily from study onset for 4 months. Compliance with product consumption during the study period was checked by regular telephone calls and weekly collection of used and unused packets. The three products had the same flavor and nutritional value per 100 g of product [1570 kJ or 369 kcal, 1.7 g of protein, 13 g of carbohydrate, 17 mg of vitamin C and 1.5 mg of vitamin E (17% and 15% of RDA, respectively)]. All children were examined at the pediatric outpatient department of San Cecilio University Hospital at the beginning and end of the study, and direct contact was made with the family every month to confirm compliance and to provide a 1 month supply of the product.

Socioeconomic interview, lifestyle, medical history and diet

Parents were interviewed on their socioeconomic status, lifestyle, family medical history and their children's health. Dietary intake was assessed by means of a food frequency questionnaire and analyzed using Nutriber Software® (Funiber Foundation, Barcelona, Spain).

Anthropometric measurements and Tanner stages

Body weight was measured using an electronic scale and standing height using a standard wall-mounted stadiometer (Holtain®). Body mass index (BMI, kg/m^2) (17) was calculated and converted into standard deviations for BMI (SDS-BMI) using Cole's LMS method (18). Other measurements included circumferences, diameters and skinfold thickness were also obtained. Pubertal stage was assessed by means of a self-report instrument consisting of schematic drawings and written descriptions of five stages of secondary sexual characteristics in two separate dimensions (female breasts and pubic hair) based on Tanner's sexual maturity scale (19). The single individual score, which can range from I (prepubertal) to V (adult) (20), was computed by averaging the two ratings.

Physical activity assessment and fitness tests

Mean weekly physical activity and sedentary levels were determined by means of a questionnaire. Three fitness tests were used from the EUROFIT Battery (21), which has been validated and standardized by the European Council. These were the Sit and Reach test, the Handgrip test and the Course-Navette test for evaluation of maximum oxygen consumption.

Biochemistry

Blood was drawn into Vacutainers® by specialists, and plasma and serum were obtained by centrifugation and immediately frozen at –80°C until analysis. Plasma triacylglycerols, total cholesterol, LDL-cholesterol, HDL-cholesterol and calcium were measured by colorimetry using a commercial kit (Roche, Neuilly sur Seine, France); serum 25-hydroxyvitamin D concentration was estimated by a radioisotope method using a Biosource International kit (Camarillo, CA, USA); thyroid stimulating hormone (TSH) and free thyroxine (FT4) were determined using an electrochemoluminiscence immunoassay (ECLIA, Roche); plasma urea, uric acid and creatinine were quantified by colorimetry using Roche commercial kits.

Bone metabolism markers Insulin-like growth factor-1 (IGF-1) was determined by immunoradiometric assay (IRMA DSL-2800, IRMA Active, Webster, TX, USA), and intact parathormone (iPTH) using a Roche ECLIA.

Bone formation biomarkers Total alkaline phosphatase (tALP) in plasma was quantified by colorimetry (Roche) according to the manufacturer's instructions. Bone alkaline phosphatase (bALP) was measured by IRMA using a Roche kit. Acid phosphatase and non-prostatic acid phosphatase and intestinal, hepatic, macromolecular and variant isozymes were determined by kinetic colorimetry (Roche). Osteocalcin was determined by RIA (N-tact® Osteo SP IRMA, Diasorin, Saluggia, Italy).

Bone resorption biomarkers Tartrate resistant acid phosphatase (TRAP) was measured by enzyme-linked immunosorbent assay (ELISA) (Bone TRAP® assay, Immunodiagnostic Systems, Boldon, UK) and type I collagen carboxy-terminal telopeptide (CTX) by ECLIA (Roche).

Statistical analysis

SPSS software (version 14.0, Chicago, IL, USA) was used for statistical analyses. A descriptive analysis was performed, yielding mean, standard deviation (SD), minimum and maximum values and frequencies. The Shapiro-Wilk test of normality was first applied and non-normal variables were transformed appropriately. A general linear model for repeated measures was performed with an intra-subject factor (T0=initial time; T1=final time after dietary intervention) and an inter-subject factor (type of gelatine product consumed). Bonferroni correction was applied for post hoc multiple comparisons. Pearson's correlation coefficient was used for Gaussian variables and Spearman's test for non-Gaussian variables. A value of $p<0.05$ was considered statistically significant.

Results

No gender differences were found in the study parameters and therefore all data are presented as pooled. The study was completed by 60 (36 boys and 24 girls) of the 71 initially enrolled children, with 6 children in G-I, 1 in G-II and 4 in G-III failing to comply with intake. The final sample comprised 18 children receiving placebo (G-I), 20 receiving gelatine (G-II) and 22 receiving gelatine supplemented with calcium (G-III) (Figure 1).

Baseline characteristics

At baseline, the 60 children who completed the study had a mean weight of 37.05±8.75 kg, height of 139.9±9.35 cm and BMI of 18.77±2.97 kg/cm^2. The 36 boys had a weight of 36.6±8.4 kg, height of 139.5±9.4 cm and BMI of 18.7±2.9 kg/cm^2. The 24 girls had a weight of 37.7±9.4 kg, height of 134.6±9.5 cm and BMI of 18.9±3.2 kg/cm^2. All participants had a Tanner stage of I-II (pre-pubertal). Baseline characteristics did not significantly differ among the groups or between genders (Table 1). Physical activity out of school hours was never practiced by 30% of the children and was practiced once or twice a week by 34% and three or more times a week by 27% of the children.

Diet analysis

Table 2 lists the mean intake of nutrients at baseline, with no significant differences among the groups. The dietary intake of nutrients did not differ between the beginning and end of the study. Only 43% of the children had an adequate intake of kilocalories (<2450 kcal/day) according to Spanish RDA values (22). All children had an excessive intake of total fat (>30%/day) and saturated fatty acids (>8 g/day), and 80% had a high dietary intake of cholesterol (<300 mg/day). Dietary deficiencies were found for vitamin D (<5 μg/day) in 35%, for calcium (<800 mg/day) in 25%, for iodine (<90 μg/day) in 76%, for phosphorus (<1200 mg/day) in 12% and for zinc (<10 mg/day) in 14% of the children.

Fitness tests

Handgrip test scores were higher for the right (17.1±4.4 kg) than for the left (16.5±4.3 kg) hand. There were no statistical differences between the sexes, indicating that the boys had not attained the muscular development that usually results in a higher score for males. The flexibility test showed a mean of 20.9±5.7 cm for the girls versus 15.4±5.8 cm for the boys ($p<0.05$). No significant differences in handgrip or flexibility test results were found among groups or between the start and end of the study period. The mean estimated maximum oxygen consumption (Course-Navette test) was 46.99±5.2 mL of O_2 per kg of body weight per min.

Anthropometric measurements

Among the under-9 year olds, 20% and 5% of the boys and 31.6% and 15.8% of the girls were overweight and obese, respectively. Among the over-9 year olds, 31.8% and 9.1% of the boys and 11.1% and 0% of the girls were overweight and obese, respectively. There were no significant differences among the groups in weight, height or BMI at the beginning or end of the study. Groups did not significantly differ in diameter and circumference measurements during the study.

Biochemical analysis

No significant differences in plasma urea, uric acid, creatinine, TSH, FT_4, acid phosphatase and non-prostatic acid phosphatase, and intestinal, hepatic, macromolecular and variant isozymes were observed. Baseline lipid profiles for the children were as follows (mg/dL) (SI units nmol/L: conversion factor for cholesterol: 0.0259 mmol/L; conversion factor for tryglicerides: 0.0113 mmol/L). Triacylglycerols: G-I, 112±42; G-II, 80±33; G-III, 101±47; total cholesterol: G-I, 171±27; G-II, 172±25.5; G-III, 175±31; LDL-cholesterol: G-I, 88±29; G-II, 92±21.5; G-III, 94±25; HDL-cholesterol: G-I, 61±9; G-II, 62±14; G-III, 60±9, with no significant differences among the groups at the beginning or end of the study. At study commencement, calcium (G-I 9.7±0.5, G-II 9.8±0.4, G-III 10.0±0.4 mg/dL) and 25-hydroxyvitamin D (G-I 32.5±15.1, G-II 28.7±13.6, G-III 28.5±12.7 ng/dL) did not significantly differ among the groups, nor did they differ at the end of the study.

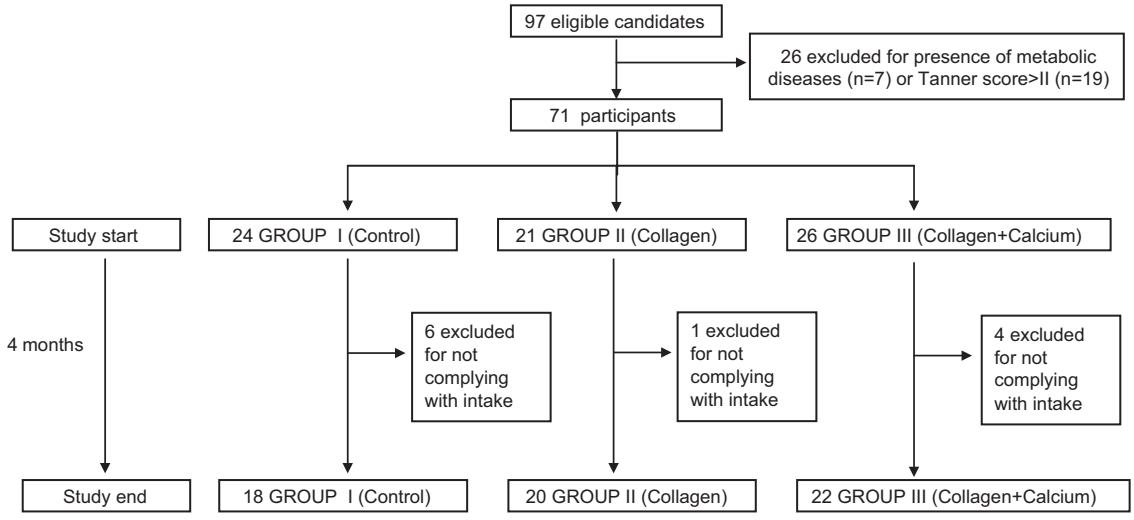

Figure 1 Flow of participants in the study.

Table 1 General characteristics of the study population by gender and study group.

	Group 1		Group 2		Group 3	
	Males	Females	Males	Females	Males	Females
Number, n	9	9	15	5	12	10
Age, years	9.7±2	9.3±1.1	9.2±1.1	10.0±1.4	9.25±1.4	9.4±1.0
Height, cm	140.3±11.5	140.1±7.8	138.8±6.8	142.2±11.5	139.7±11.5	140.25±10.8
Weight, kg	37.7±11.2	36.4±7.5	36.1±7.2	35.2±6.2	36.4±8.1	40.1±12.3
BMI, kg/m^2	18.8±3.4	18.6±2.9	18.7±2.8	17.4±1.4	18.5±2.7	20.0±3.9
Skinfold thickness, mm						
Triceps	15.0±6.3	14.6±5.7	14.1±4.6	14.2±4.6	14.4±5.0	15.0±5.4
Subscapular	9.7±5.3	9.9±5.6	8.7±4.4	8.3±4.3	8.9±5.2	10.0±6.6
Abdominal	14.6±8.8	14.5±7.8	13.4±7.3	13.4±6.5	14.4±7.6	16.5±9.1
Suprailiac	9.5±5.9	9.0±5.2	8.2±3.6	8.5±3.9	9.6±5.4	11.2±7.2
Circumference						
Thorax, cm	71.5±7.3	71.1±7.7	69.8±4.5	70.35±5.4	70.3±8.0	71.6±8.0
Waist, cm	64.3±7.6	64.8±7.5	63.7±5.9	63.8±6.9	63.6±7.2	66.6±9.13
Hip, cm	75.7±8.8	76.3±8.5	74.7±6.7	75.7±7.2	76.6±9.0	78.9±10.1
Physical activity, h/week after school	1.0±0.7	0.7±0.7	1.1±0.8	0.6±0.5	1.2±0.9	0.9±0.7
Tanner stage						
I, %	44	44	60	40	50	40
II, %	56	56	40	60	50	60

Table 2 Daily dietary intake of nutrients at baseline.

Daily intake	G-I	G-II	G-III	Spanish RDA
Energy, kcal	2317.9±437.0	2331.3±428.25	2119.8±414.6	2200–2500
Protein, g	33.0±18.1	34.1±26.0	35.2±20.2	30–36
Fat, g	127.1±29.3	119.8±28.3	111.0±27.7	70–80
Saturated fatty acids, g	28.7±7.9	24.7±6.5	21.7±7.8	8–10
Monounsaturated fatty acids, g	66.7±16.1	59.0±18.2	54.7±18.3	11–13
Polyunsaturated fatty acids, g	12.8±5.1	13.5±4.6	11.9±4.9	5–7
Cholesterol, mg	271.7±91.0	254.6±52.2	245.3±78.0	<300
Carbohydrates, g	213.7±48.2	233.7±51.1	208.0±40.8	250–300
Fibre, g	15.4±4.5	15.8±4.2	15.0±4.0	>25
Calcium, mg	1122.6±281.4	1052.5±321.1	949.2±249.0	800
Magnesium, mg	299.6±93.5	297.3±45.9	276.8±61.3	250
Iron, mg	15.0±4.8	19.4±13.9	17.3±8.3	9
Zinc, mg	9.05±2.9	11.4±9.7	10.5±6.6	10
Iodine, µg	59.9±21.8	53.0±19.4	50.2±26.0	90
Vitamin B1, mg	3.7±3.9	3.5±3.7	3.8±2.9	1
Vitamin B2, mg	1.9±.7	2.0±.6	1.9±.5	1.5
Vitamin B6, mg	3.0±3.8	2.8±1.9	4.6±7.8	1.4
Vitamin B12, µg	7.1±2.4	7.6±2.7	7.5±3.1	1.5
Folic acid, µg	267.7±80.6	254.6±89.9	214.7±72.5	100
Niacin, mg	23.5±8.3	24.0±5.8	23.6±7.5	13
Vitamin C, mg	123.1±65.2	111.5±51.3	113.5±72.6	55
Retinol, µg	820.5±281.3	808.2±281.5	660.5±256.5	400
Vitamin D, µg	5.1±3.6	6.3±3.6	4.2±3.1	5
Vitamin E, mg	16.0±3.75	15.7±5.9	15.2±5.4	5

Bone markers

Table 3 shows the changes in mean bone biomarker values. There was a significantly greater increase in serum IGF-1 in G-III than in G-II ($p<0.01$) or G-I ($p<0.05$) during the study period, and a significantly greater increase in plasma tALP in G-III than in G-I ($p<0.05$). Serum bALP behavior significantly ($p<0.05$) differed between G-II and G-I, increasing in the former and decreasing in the latter. Plasma TRAP behavior significantly differed between G-I and G-II (1.6±4.2 vs. –1.2±4.0, $p<0.01$) and between G-II and G-III (–1.2±4.0 vs. –1.5±3.4, $p<0.05$). Plasma CTX behavior in G-III (–0.20±0.25) significantly differed ($p<0.05$) from that in G-I (0.07±0.430) and G-II (0.03±0.44).

Discussion

Recent studies have demonstrated that nutrient intake in the diet influences bone remodeling (14, 23), including calcium (24), phosphorus (25), vitamins C and D (26) and suitable intake of animal protein (27). Bone continues to develop until the age of 35 years, and a high bone density must be attained to prevent bone loss in old age (28). The positive effect on bone acquisition of habitual physical activity during childhood and adolescence, especially weight and strength training, has been demonstrated by various groups (29–31). A lack of physical activity in early stages could predispose to bone diseases, as well as overweight and obesity. BMI is used to evaluate overweight and obesity states in children and adolescents (32) and in the general population (33). Approximately 30% of the children in the present study were overweight and almost 10% were obese, similar to data published by WHO on obesity prevalence in Spain (34).

In children, assessment of the effects of dietary factors in this context has primarily focused on the amount of calcium required for optimal bone accrual, because the skeleton matures at a relatively early age (35). However, the calcium and mineral contents of the skeleton seem to be markedly influenced by nutrients other than calcium, specifically protein (36) and alkalizing minerals (37), which are increasingly described as playing a major role. We hypothesized that a diet high in calcium and protein would minimize bone resorption during weight loss compared with oral calcium and collagen supplementation. Schwarz et al. treated rats with collagen and apatite and observed an increase in bone formation and alkaline phosphatase during the 3 weeks of the intervention (38). Various authors have reported that collagen proteins are needed for correct formation of bone structure (39). Whiting et al. investigated the relationship between bone mineral density in adult men and their intake of calcium, protein, phosphorus and potassium (27). They concluded that a moderate protein (1.2 mg/kg) diet plentiful in potassium and phosphorus is beneficial for maintaining bone mineral density in male adults when calcium intake is adequate. Vatanparast et al. showed that when calcium intake is adequate, protein intake has a beneficial effect on the bone mass of young adult females (40). One study evaluated the effects of collagen type I and its role in bone metabolism, and found increased bone regeneration and serum alkaline phosphatase in treated individuals (41). Hydrolyzed gelatine or collagen (with essential amino acids such as arginine) has long been used in drugs and foods and is generally recognized as a safe food product by regulatory agencies and is a easy way to administer an extra amount of protein to children (42). Clinical studies have indicated that daily intake of 10 g of hydrolyzed collagen reduces pain in patients with osteoarthritis of the knee or hip and produced greater inhibition of bone collagen breakdown compared with calcitonin (16). The effects of dietary protein on bone health are paradoxical and seem to depend on the age, health status and usual diet of the population. Prospective studies in the USA showed greater bone losses in elderly men and women with a mean protein intake of 16–50 g/day than in those with a higher intake (7).

Changes in bone modeling have been observed during four growth periods: infancy, pre-puberty, puberty and post-

Table 3 Serum bone biomarkers at the start and end of the study period.

	Study start			Study end			Change		
	G-I	G-II	G-III	G-I	G-II	G-III	G-I	G-II	G-III
IGF-1, ng/mL	190.5±92.4	177.7±84.3	217.3±106.3	265.1±129.3	257.9±143.9	382±186.7	73.5±160.2[a]	59.2±59.0	152.5±134.3[b]
iPTH, pg/mL	60.6±31.3	60.7±25.65	56.9±24.3	62.9±24.0	61.45±38.6	58.7±34.0	0.9±27.45	–1.3±30.4	0.6±27.7
tALP, IU/L	261.1±64.4	265.8±68.1	248.5±59.9	253.1±51.3[c]	260.3±71.0	254.85±64.4	–6.6±32.1	–0.5±31.2	0.2±28.7
bALP, µg/mL	185.5±51.9	204.1±42.8	198.5±52.0	179.5±39.0	204.8±42.9	195.2±61.1	–28.6±29.9[e]	2.35±42.6	–5.7±32.0
Osteocalcin, ng/mL	11.3±6.4	10.6±4.7	10.6±5.1	9.6±11.8	7.7±7.6	10.8±6.8	–2.1±14.3	–4.0±8.1	0.4±10.1
TRAP, IU/L	6.9±2.9	7.6±2.9	7.2±2.7	8.2±4.2	6.5±3.8	5.7±2.8	1.6±4.2[d]	–1.2±4.0	–1.5±3.4[e]
CTX, ng/mL	1.25±0.33	1.205±0.34	1.26±0.37	1.33±0.56	1.23±0.41	1.06±0.37	0.07±0.43[a]	0.03±0.44	–0.20±0.25[e]

[a]$p<0.05$ placebo vs. collagen. [b]$p<0.01$ placebo vs. collagen. [c]$p<0.05$ placebo vs. collagen with calcium. [d]$p<0.05$ collagen vs. collagen+calcium. [e]$p<0.01$ collagen vs. collagen+calcium. CTX, type I collagen carboxy-terminal telopeptide; IGF-1, insulin-like growth factor-1; iPTH, intact parathormone; bALP, bone ALP; tALP, total alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase.

puberty. Because all children in the present study were in the pre-pubertal stage and no general gender differences were observed, data were pooled to analyze the effect of the different dietary supplements on bone remodeling. Available data suggest that biochemical markers of bone remodeling may be useful in the clinical investigation of bone modeling in healthy and diseased children (43, 44). In this investigation, products of osteoblast activity were measured as bone formation markers (tALP, bALP, osteocalcin, IGF-1) and products of osteoclast activity (CTX and TRAP) as bone resorption markers. The increase in IGF-1 was significantly greater in G-III compared to G-I and G-II. IGF-1 is the main determinant of bone growth and mineral content (45) and stimulates osteoblasts in vitro, with a possible effect on skeletal muscle mass. Kerstetter et al. evaluated the effect of dietary protein on bone modeling markers in 16 young healthy women who received a well-balanced diet for 2 weeks, with moderate amounts of calcium, sodium and protein, followed by a 4-day experimental diet with low, medium, or high levels of protein (13). No difference in osteocalcin or bALP was found between those receiving the low-protein and high-protein diets, suggesting that bone resorption was increased with no compensatory increase in bone formation. In the present study, the group receiving collagen and calcium showed no modification in osteocalcin, an increase in bALP and a significant decrease in bone resorption biomarkers (TRAP and CTX). Therefore, the intake of collagen and calcium may have influenced bone modeling in these children.

The main and novel finding of this study is that administration of collagen in early stages of life may represent a preventive therapy against bone disease. Previous investigations have focused on the role of collagen in treating these diseases. The main study limitations are the small sample size and the failure to evaluate the osteoarticular impact of physical activity. In future studies of this type, dual-energy X-ray absorptiometry should be used to evaluate any changes in bone densitometry.

In summary, collagen may play a major role in optimal growth and development at critical stages of life and may have a positive influence on bone remodeling. Our results indicate that prolonged dietary collagen intake seems to stimulate bone formation during important periods of growth and development, influencing both bone formation (bALP) and bone resorption (TRAP and CTX) biomarkers. Further studies are warranted to elucidate the role of nutrition and physical activity in bone metabolism during early stages of life to ensure optimal bone peak mass and quality, thereby reducing the risk of osteoporosis, arthritis and other bone diseases in later life.

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