Colostrum is a complex mixture of bioactives that promotes neonate growth. Studies show that it contains components capable of promoting bone formation and inhibiting bone resorption. Although many colostrum-based nutritional supplements have been developed as growth promotants, few studies have investigated their functional effects. A bovine colostrum 1–30 kDa fraction, Growth Protein-Colostrum (GP-C), was administered to juvenile rats as a dietary supplement to determine effects on growth and development. GP-C enhanced the growth and mineralization of the femur as evidenced by increased serum osteocalcin and bone mineral density. Increased levels of serum growth hormone and insulin-like growth factor-1 suggest that the mechanism of enhanced growth is partially controlled by endocrine factors. GP-C was also found to increase osteoblast proliferation in vitro, a finding that indicates a possible mechanism of action of GP-C, but further studies are required. Based on our findings, we hypothesize that a colostrum-based dietary supplement enhances bone growth and development in humans.

Key words: colostrum; growth protein; bone growth

It has long been known that cow’s milk whey protein contains several components, particularly in the basic protein fraction, capable of promoting bone formation and inhibiting bone resorption.1–3) Studies have identified proteins with growth-promoting activity as assessed in an osteoblastic cell model.6) Compared with mature milk, colostrum contains higher levels of growth-promoting proteins, and bovine colostrum is a convenient source of these proteins in that it is obtainable in large quantities.5)

Data from an in vitro study examining the effects of a basic protein fraction isolated from bovine whey protein on osteoblastic MC3T3-E1 cells suggest that basic protein plays an important role in bone formation by activating osteoblasts,6) but the primary mechanism of action might be through inhibition of osteoclast-mediated bone resorption.1) Whatever the precise mechanism, dietary supplementation with milk basic protein has been found to produce a significant increase in bone mineral density (BMD) in healthy adult women,1) and it has also been found to promote bone formation and to suppress bone resorption in healthy adult men.7) The experiments of the current investigation, in contrast, were aimed at determining whether the colostrum fraction GP-C would increase animal size and longitudinal bone growth.

The growth and development of neonate mammals is finely controlled by a complex cascade of regulatory molecules including cytokines, hormones, and growth factors. Longitudinal bone growth is achieved via chondrocytes of the growth plate multiplying and elongating in the proliferative and hypertrophic zones respectively. These cells lay down a cartilage matrix and then undergo apoptosis.

Further removed from the hypertrophic zone, osteoclasts hydrolyse cartilage and osteoblasts develop new bone matrix, including osteocalcin and osteonectin. In addition to direct hormonal control of these cells, the osteoblasts themselves secrete factors that modify the activity of growth hormones, such as insulin-like growth factor-1 (IGF-I), and enzymes, such as bone-specific alkaline phosphatase (BALP), which is known to stimulate bone mineralization.8)

IGF-1 influences bone density and is a marker of overall growth hormone (GH) status.9) It is stored in the bone matrix and is released during active bone resorp-
tion, when released IGF-I is available to recruit osteoblasts to the bone surface, playing a critical role in bone remodeling.

Osteocalcin (OST), also known as Gla protein, is unique to bone tissue and dentin and is the most abundant non-collagenous protein of bone. It is synthesized by osteoblasts and is incorporated into the extracellular matrix, although a small fraction of newly synthesized OST is released into circulation, where it can be measured. Serum OST correlates well with skeletal growth at puberty and increases in conditions characterized by increased bone turnover (e.g., hyperparathyroidism, hyperthyroidism, Pagets’ disease, and acromegaly). For these reasons, OST is routinely used as a specific, sensitive marker of bone formation.

IGF-1 is also known to stimulate gastrointestinal (GI) development. The active components in colostrum have been found to stimulate growth of the GI tract. Colostrum provides greater GI growth than mature milk in newborn pigs and newborn rats, enabling the infant to absorb more nutritional value from the diet earlier and indirectly influencing overall growth rates. The mechanism is unknown, but the effect might be due to the action of growth-promoting molecules in colostrum, stimulating IGF-I secretion from the liver.

Although many colostrum-based nutritional supplements have been developed as growth promotants, few studies have investigated their functional effects. A 1–30 kDa protein fraction with potential growth-promoting activity (Growth Protein-Colostrum, GP-C) has been prepared from colostrum. Since the extraction process is unique, the extract’s nutritional value has not previously been evaluated. Ultrafiltration has been used to fractionate whey proteins previously.

The objective of this study was to ascertain the effect of GP-C on bone growth and to elucidate the mechanisms by which GP-C, administered as a dietary supplement, enhances bone development. This involved investigating the in vivo effects of various doses of GP-C versus control on the physical and biochemical assessments of bone growth and to elucidate the mechanisms by which GP-C, administered as a dietary supplement, enhances bone development.

Materials and Methods

Materials. The GP-C fraction was prepared using a proprietary process owned by Seperex, Ltd. (New Zealand). An outline of the process is described here. Selected commercial-grade bovine colostrum (typically from the first 5–6 milkings) was skimmed, pasteurized, ultrafiltered, and spray-dried to produce a high-protein Colostral Milk Protein Concentrate (CMPC). The CMPC was subsequently reconstituted and depleted of major non-whey proteins by diafiltration (30 kDa membrane). The permeate was depleted of low molecular weight species by ultrafiltration/diafiltration (1 kDa membrane), followed by blending. The majority of proteins present in the GP-C were within the 1–30 kDa range (data not shown). The total protein, lactose, ash, moisture, and fat content of the finished product are shown in Table 1.

Animal study. Twenty-eight male, white 3 weeks old Sprague-Dawley rats weighing 50–56 g were randomly allocated into four groups of seven animals. Rats were approximately 10 weeks old at the conclusion of the experiment, which was performed in accordance with National Institutes of Health animal-care guidelines.

Diet. After a 1-week adaptation period on normal feed, the groups were fed different dosages of GP-C in high protein diets for the following 6.2 weeks (weeks 0 to 6.2). The total GP-C plus casein comprised 20% of the diet mixture, as described in Table 2. Food and water were provided without limitation for the duration of the experiment. Casein has been used as a control protein in other studies measuring bone growth in juvenile rats. Behavioral observations were concluded 6.2 weeks from the start of the diet, and immediately following this, the rats were sacrificed and various physical and biochemical (serum proteins) assessments were made.

Physical assessments. Assessments included body weight, bone weight, bone length, and bone mineral

| Table 1. Protein, Lactose, Ash, Moisture, and Fat Content of GP-C |
|-----------------|---------|---------|---------|---------|
| % (w/w) |
| Protein (TN) × 6.38 | 66.3 |
| Lactose | 13.1 |
| Ash | 4.6 |
| Moisture | 5.8 |
| Fat | 5.2 |

TN, Total Kjeldahl nitrogen

| Table 2. Diet Composition Expressed as Percentages of the Total |
|-----------------|---------|---------|---------|---------|
| % GP-C | 0 | 0.05 | 0.5 | 5 |
| Casein | 20.00 | 19.95 | 19.50 | 15.00 |
| GP-C | 0.00 | 0.05 | 0.50 | 5.00 |
| Corn starch | 39.75 | 39.75 | 39.75 | 39.75 |
| α-corn starch | 13.20 | 13.20 | 13.20 | 13.20 |
| Sugar | 10.00 | 10.00 | 10.00 | 10.00 |
| Cellulose | 5.00 | 5.00 | 5.00 | 5.00 |
| Soybean oil | 7.00 | 7.00 | 7.00 | 7.00 |
| T-butylhydroquinone | 0.0014 | 0.0014 | 0.0014 | 0.0014 |
| Cystine | 0.30 | 0.30 | 0.30 | 0.30 |
| Choline HT | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin mixa | 1.00 | 1.00 | 1.00 | 1.00 |
| Mineral mixb | 3.50 | 3.50 | 3.50 | 3.50 |

a310025 AIN-VX vitamin mixture.
b210025 AIN-936 mineral mixture.

GP-C, growth protein-colostrum
All animals were weighed and sacrificed, and the right and left femurs of each animal were removed. Each femur was cleaned by scraping, and dried at 75°C for 3 h, and its length and weight were measured. The BMD of each femur was measured by an XR series X-ray densitometry system (Norland, Fort Atkinson, WI).

**Serum assessments.** At the conclusion of the experiment, blood was taken from each rat, and GH, IGF-1 and OST levels were measured and compared between groups. GH levels in the serum were measured by immunoenzymometric (IRMA) assay using a commercial kit according to the manufacturer’s instructions (DSL-1900 Active Kit; DSL, Webster, TX) and hormone levels were measured using a Cobra 5010 R–Counter (Packard Instruments, Downers Grove, IL). IGF-1 levels were also measured by an IRMA method with a commercial OCTEIA Rat IGF-1 kit according to the manufacturer’s instructions (IDS, Fountain Hills, AZ) and the optical densities of the samples were measured at 450 nm using an ELx800 microplate reader (Bio-Tek Instruments, Winooski, VT). OST was quantified by ELISA utilizing two rat osteocalcin antibodies (BTI Rat Osteocalcin EIA kit, cat BT-490; Biomedical Technologies, Stoughton, MA).

In **vitro** experiment. The ability of GP-C to influence the proliferation of osteoblasts (bone-forming cells) was investigated by an *in vitro* osteoblast proliferation assay. Three concentrations of GP-C (1, 10, and 100 µg/ml) and a control containing no GP-C were used. Osteoblast-like MC3T3-E1 cloned cells (ATCC CRL-2593) were cultured and incubated with and without GP-C for 48 h, and proliferation was measured by standard MTT colorimetric assay. Results were expressed as percentages of control (control = 100%).

**Rationale for assessments.** Serum GH, IGF-1, and OST have all been found to correlate with bone growth in previous studies.9,10,16,17) OST is a specific, sensitive marker of bone formation, and turnover and is more selective than BALP, since it is localized to bone tissue (aside from a small amount present in the blood stream).10) Assessment of BMD was performed to determine whether potential growth effects would be associated with alterations in bone strength.

**Statistical analyses.** In **vivo** physical and serum assessments are reported as mean change from baseline ± one standard deviation (SD), and statistical significance of the means for each of the three experimental groups was compared with the control group by Student’s *t*-test (significance set at 0.05). In **vitro** osteoblast proliferation was expressed relative to control (no GP-C). Control means were compared with each of the three experimental GP-C group means by Student’s *t*-test (significance set at 0.05).

**Results**

**Animal**

**Physical assessments**

At the conclusion of the study (week 6.2), the average weight gain of all groups fed GP-C was greater than that of the controls (Fig. 1, insert). However, only the weight gain of the rats fed the highest dose of GP-C (5%) was statistically significant as compared to the controls...
The average weight gain correlated with the dose of GP-C (Fig. 1). The femurs from rats fed 5% and 0.5% GP-C were on average heavier than those of controls (0.861 g, \(\text{p} < 0.05\), and 0.829 g, \(\text{p} < 0.001\), versus 0.735 g respectively) (Fig. 2), and longer than those of the controls (38.37 mm and 38.05 mm, versus 36.88 mm respectively; \(\text{p} < 0.01\)) (Fig. 2). The fact that the femurs of the rats fed higher doses of GP-C were both longer and heavier than the controls suggests that bone growth did not occur at the expense of bone strength. This conclusion is supported by the BMD results, in that the rats fed the highest dose of GP-C (5%) had a significantly higher BMD than the controls (\(\text{p} < 0.05\), t-test). Data are means +/- 1 SD.

Serum assessments

The serum GH level was significantly higher in the 5% GP-C group than in the control (\(\text{p} < 0.01\)) (Fig. 4A). The increased GH was reflected by increases in IGF-1 in the serum of rats fed 0.5% or 5% GP-C as compared to the controls (\(\text{p} < 0.05\) and \(\text{p} < 0.01\), respectively) (Fig. 4B). The 5% GP-C and 0.5% GP-C groups also showed significantly higher mean serum OST levels than the controls (\(\text{p} < 0.05\) and \(\text{p} < 0.001\), respectively) (Fig. 4C).

In-vitro

Osteoblast proliferation was significantly enhanced in a medium containing GP-C relative to control (0% GP-C) (1 \(\mu\)g/ml, \(\text{p} < 0.05\); 10 and 100 \(\mu\)g/ml, \(\text{p} < 0.01\)) (Fig. 5).

Discussion

At the conclusion of our study, rats fed the highest dose of GP-C (5%) weighed significantly more than the control rats. GP-C also produced dose-dependent increases in bone weight and length, indicating that GP-C promoted longitudinal bone growth.

Data on the optimal levels of dietary protein for maximal growth in rats are limited. The US National Research Council’s Committee on Animal Nutrition suggests that the level of casein protein for optimum growth of rats is 23% for 100% of maximum growth.\(^{18}\) This level decreases to 20% casein protein when allowance is made for the addition of 0.3% cystine to the diet since cystine and methionine are the limiting amino acids in casein. In our study, we used a control diet consisting of 20% casein plus 0.3% cystine, so that the amino acids were very close to the optimal level, at which the growth rate is independent of the dietary protein concentration.

In the control group, the growth rates slowed faster than in the other groups as the animals approached adult size. GP-C administered as a dietary supplement appears to have delayed the onset of the growth rate plateau in a dose-dependent manner. The normal age-related decline in bone growth rates is due primarily to a decrease in the rate of growth-plate chondrocyte proliferation. In vivo studies suggest that the decrease occurs because growth-plate chondrocytes have a finite proliferative capacity.\(^{19}\) Eventually, when the chondrocytes’ proliferative capacity is exhausted, growth ceases and the growth plate is replaced by bone (a process known as endochondral ossification or epiphyseal fusion).\(^{19}\) This programmed
senescence of chondrocytes can be delayed or accelerated, causing more or less growth to occur. It appears to be controlled by a local cellular or molecular mechanism within the growth plate, but external mechanisms affecting chondrocyte proliferation might include a direct effect of GH on the growth plate.

One hypothesis as to the stimulation of bone growth seen with colostrum dietary supplementation is that a factor or factors present in the colostrum stimulate endogenous GH release by the pituitary. GH influences bone growth directly by stimulating differentiation of chondrocytes and indirectly through stimulation of IGF-1 secretion by the liver. IGF-1, produced both locally and from blood, stimulates proliferation of chondrocytes, resulting in bone growth. Indeed, new bone formation, via increased osteoblastic activity, is indicated by the significant elevation in serum OST levels that were seen with the two highest doses of GP-C.

In our study, growing rats fed GP-C, at doses of 0.5% and 5%, had significantly greater bone growth and mineralization than the control group indicating that dietary GP-C can stimulate enhanced bone development. The significant increase in basal levels of GH and IGF-1 in the serum of rats with GP-C dietary supplementation as compared to controls suggests that the mechanism of increased femur growth is at least partially controlled by endocrine factors.

The effect of GP-C on osteoblast proliferation demonstrated in vitro in this study indicates a possible mechanism for increased mineralization of bone. Given the effects of GP-C on biochemical markers of bone modeling in the present study, further investigation is warranted to determine whether GP-C retards or prevents the development of bone remodeling disorders during adulthood. For example, it would be interesting

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**Fig. 4.** Serum Levels of Growth Hormone, IGF-1 and Osteocalcin for Control and GPC Groups.

In general, rats fed GP-C had higher serum levels of growth hormone (A), insulin-like growth factor-1 (IGF-1; B) and osteocalcin (C) as compared to controls. In particular, rats fed 5% GP-C had significantly higher levels of all three peptides relative to controls ($^*p < 0.05$ or $^**p < 0.01$, t-test) whereas rats fed 0.5% GP-C had higher levels only of IGF-1 and osteocalcin ($^*p < 0.05$ or $^***p < 0.001$ respectively, t-test). Data are means $+/-$ 1 SD.

**Fig. 5.** Effect of Various Levels of GP-C on Proliferation of Osteoblast-Like Cells.

GP-C had a direct affect upon the proliferation of osteoblast-like cells in-vitro. GP-C dissolved in culture medium at levels of 0 (control), 1, 10, and 100 µg/ml was added to cultured osteoblast-like cells (MC3T3-E1) and incubated for 48 h. Cell proliferation was measured by MTT colourimetric assay. Data are means $+/-$ 1 SD expressed as percent of control values ($^*p < 0.05$, $^**p < 0.01$ versus control; t-test). Data are means.
to determine whether GP-C affects bone turnover and remodeling in ovariectomized rats in order to achieve insight into the use of GP-C as a potential preventative nutritional therapy for osteoporosis. The notion that dairy-derived extracts can have beneficial effects on BMD and strength in adult mammals is well supported in the literature.\textsuperscript{22,23)\textsuperscript{\textcopyright}}

In summary, our findings indicate that GP-C contains one or more functional ingredients that promote bone development in juvenile rats, and they lead us to hypothesize that a colostrum-based functional food enhances bone growth and development in humans. Further in vivo and in vitro experiments are required to identify the exact mechanisms of action of GP-C.

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