Journal of Science & Cycling
Breakthroughs in Cycling & Triathlon Sciences

OPEN ACCESS

Original Research

Twelve Week Calcium Collagen Chelate or Calcium plus Vitamin D Supplementation Does Not Affect Bone Metabolism in Training Cyclist

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Received: 14 July 2020; Accepted: 22 April 2022; Published: date

Abstract: The purpose of the present study was to determine whether 12 weeks of calcium collagen chelate (CCC) supplementation during habitual training would affect body composition, bone mineral density (BMD), and biomarkers of bone metabolism in competitive cyclists. Twenty trained (maximal aerobic capacity > 50 mL/kg/min, mean training volume: 28 h/wk) male cyclists performed maximal exercise testing and 40-km time trials (TT) on an electronically braked cycle ergometer. BMD of the whole body, lumbar spine (L1-L4), and both hips were measured via dual-energy X-ray absorptiometry (DXA). The cyclists were assigned to one of two groups: 1) 6 g/d of CCC with 600 mg calcium and 400 IU vitamin D or 2) a control treatment (CON) composed of an inert compound with equivalent calcium and vitamin D concentrations to CCC. Two-way repeated measures ANOVA and Pearson product-moment correlations were used to determine the effects of CCC or CON supplementation on BMD, bone alkaline phosphatase (BAP), tartrate resistant acid phosphatase 5b (TRAP5b), and sclerostin (SCL); significance was accepted at $p < 0.05$. No within- or between-group differences in dependent variables were found. Significant correlations were found between weekly training volume and TRAP5b (r = 0.531), BAP and VO_2 max (r = -0.561), and BAP/TRAP5b ratio and both right/left hip BMD (r = -0.649 and r = -0.646, respectively). In conclusion, 12 weeks supplementation of CCC does not affect body composition, BMD, or biomarkers of bone metabolism in trained, competitive cyclists in comparison to equivalent amounts of calcium plus vitamin D.

Keywords: dietary supplements, bone mineral density, cycling

1. Introduction

Exercise affects the skeleton through the increased strain placed on bone during physical activity. Athletes participating in sports with the highest strain intensities (*e.g.*, gymnastics, weight lifting) have significantly higher bone mineral density (BMD) at the loaded sites (16, 21, 23) than athletes participating in non-weight bearing sports. Likewise, the osteogenic stimulus is attenuated when ground-reaction forces are reduced; this effect has been shown in the lower body of swimmers and has the potential to be even more detrimental in cyclists, who may spend more time than swimmers training with no or low impact movements (16, 21, 23). Similarly, significant differences (p = 0.04) were observed when BMD was compared in a group of age, weight and training volume matched weight-bearing athletes (whole body BMD 1.26 ± 0.03 g/cm^2) to that of non-weight-bearing athletes (low ground-reaction forces; whole body BMD 1.20 ± 0.01 g/cm^2) (24).

Dietary supplements such as calcium and/or hydrolyzed collagen have been proposed as strategies for improving bone health due to their predominance in bone tissue (4, 8, 12), but intervention strategies have yielded mixed results on BMD and biomarkers of bone turnover. In a study of trained, adult male road cyclists and triathletes, supplementation of a 1000 mg/L calcium citrate malate drink consumed 20 minutes prior to exercise significantly attenuated the increase in parathyroid hormone following a 35-km time trial (TT) (4). Although bone-specific alkaline phosphatase (BAP) and C-terminal telopeptide of Type I collagen (CTX) levels immediately post-exercise were not affected by calcium supplementation, it should be noted that mean 25-hydroxyvitamin D concentrations were relatively low (32.7 ± 9.2 ng/mL) with nine of the 20 subjects classified as insufficient (< 30 ng/mL). Compromised vitamin D status may have contributed to reduced absorption of the calcium, limiting the potential benefits of supplementation. The timing of immediate, post-exercise blood draws may have also contributed to the lack of measured response to the supplementation; alternate investigators found significant increases in CTX levels that were not evident until 30 minutes to two hours post-exercise following ingestion of high calcium (486 mg/h) mineral water during a 60-minute cycling bout (12).

Supplementation of collagen has been shown to increase BMD, particularly in trabecular bone, as well as positively influencing biomarkers of bone turnover (13). Ovariectomized rats, a model of post-menopausal osteoporosis, had significant increases in whole body BMD, cortical area, and increases in femur strength following 12 weeks of hydrolyzed collagen supplementation (13). Likewise, six months of hydrolyzed collagen supplementation (10 g/d) and calcitonin (100 U twice per week) provided significantly greater reductions in bone resorption markers as compared to calcitonin alone (1) in post-menopausal women. However, collagen hydrolysates (10 g/day) taken over 24 weeks had no effect on bone metabolism in post-menopausal women, although most subjects were not ingesting adequate dietary calcium (8).

The purpose of the present investigation was to identify if supplementation with calcium collagen chelate (CCC) during a 12-week training period would influence body composition (*i.e.*, BMD, lean mass) and biomarkers of bone turnover in trained cyclists. It was hypothesized that CCC supplementation would positively influence both BMD and all blood biomarkers. This project is novel in that CCC is a relatively new dietary formulation that has never, to our knowledge, been tested in an athletic population, but has been shown to attenuate bone loss in postmenopausal women (10). Additionally, the present study addresses limitations in previous supplementation studies by combining a CCC intervention with vitamin D supplementation, potentially improving vitamin D status which has been shown to aid with bone remodeling in men (29).

2. Materials and Methods

Subjects — Sample size estimation was determined *a priori* as a function of the significance criterion (α), the statistical power and effect size (ES). For this experiment, an effect size of 0.72 was calculated based on Barry and Kohrt (5) who examined changes in total hip BMD over a four and a half month period during a competitive season in trained cyclists. Statistical significance was set at $\alpha = 0.05$, ES = 0.72, and a statistical power of 0.80, which yielded a minimum of 10 subjects per group. Twenty-nine healthy male cyclists were initially invited to the laboratory, as it was assumed several would not meet the inclusion conditions imposed to qualify as "trained," defined as engaging in a minimum of three hours of cycling training per week.
Subjects were required to have a normal body mass index (BMI: 18.5-24.9 kg/m^2), be free of signs of symptoms suggestive of cardiovascular, pulmonary, or metabolic disease, meet the minimum volume requirement for being "trained," and have a VO_2 max of > 50.0 mL/kg/min (assessed during the first laboratory visit). Subjects training more than two days per week in any non-cycling activity within the past six months were excluded. Additionally, those who smoke cigarettes or used any prescription medications known to affect bone metabolism (*e.g.*, statins) were excluded.

All methodologies were approved by the Florida State University Institutional Review Board (HSC No. 2013.9887), and all subjects provided informed consent.

Design — This study utilized a single blinded, repeated measures, parallel experimental design during a normal 12 week training period for competitive cyclists. The independent variable for all comparisons was dietary supplement assignment (CCC with vitamin D or a calcium+vitamin D supplement). The primary dependent variables were bone density scores and markers of bone turnover.

The decision to use a control treatment (calcium+vitamin D alone) instead of a biologically inert placebo is justified as it

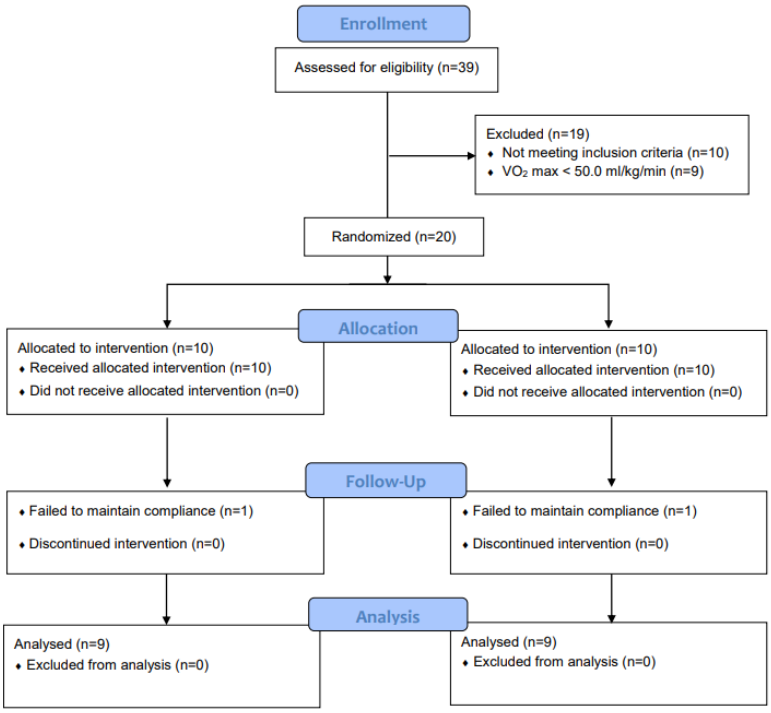

Figure 1. Flow Chart of study inclusion.

Table 1. Means and Standard Deviation for Descriptive Variables

	CCC (N=9)		CON (N=9)	
Variable	Mean±S.D.	Range	Mean±S.D.	Range
Age (years)	39 ± 11	21-54	39 ± 7	30-52
Height (m)	1.77 ± 0.08	1.68-1.95	1.82 ± 0.06	1.71-1.89
Weight (kg)	75.2 ± 10.5	65.0-99.4	78.4 ± 8.7	66.4-95.5
Body Fat (%)	18.2 ± 4.3	14.2-27.9	20.6 ± 2.8	15.6-23.8
BMI (kg/m^2)	23.9 ± 1.5	22.5-26.1	23.6 ± 1.8	20.8 ± 26.7
LBM (kg)	57.6 ± 6.1	48.3 ± 65.7	59.4 ± 7.2	50.9-74.9
Training History(yrs)	16.3 ± 11.2	4.0-30.0	14.6 ± 7.9	5.0-26.0
Training Vol. (h/wk)	10.4 ± 2.3	7.5-16.0	10.3 ± 2.5	7.0-16.0

Note. CCC = calcium collagen chelate supplement group; CON = control group; BMI = body mass index; LBM = lean body mass

decreased the chance of nutritional inadequacy as a confounding variable.

Methodology: Baseline Measures — Subjects reported to the laboratory after a three hour fast and with > 24 hours since their last strenuous bout of physical activity. After potential subjects had been screened via telephone calls to ensure eligibility, subjects were required to keep a three-day dietary log including all foods and serving sizes for use prior to the initial laboratory visit.

After resting heart rate (HR) and blood pressure (BP) measurements were made in duplicate with their means recorded for data analysis, blood was sampled via venipuncture from the antecubital space of the forearm, gently mixed and allowed to clot, centrifuged at 3500 rpm for 15 minutes to isolate serum, divided into aliquot samples and stored at -80°C for later analysis of BAP, Tartrate-resistant acid phosphatase 5b (TRAP5b), and sclerostin (SCL). BAP is an indicator of bone formation (28) while TRAP5b is a maker of bone resorption (14), and the BAP/TRAP5b ratio provides an overall index of the bone formation/resorption ratio. SCL is a regulator of bone formation, providing a marker for mature osteocyte concentration (15). All assays were performed in duplicate using enzyme linked immunosorbent assays (ELISA) according to the manufacturer's instructions (MicroVue TRAP5b EIA Kit 8033; Sclerostin High Sensitivity EIA Kit TE1023HS; BioTek Instruments, USA).

Height and body weight were measured using a stadiometer and digital scale (SECA, Birmingham, United Kingdom), respectively. Body composition was evaluated by dual energy X-ray absorptiometry (DXA; Hologic Discovery W, Bedford, MA). Total body, hip and lumbar BMD scans were performed. Before testing, subjects were asked to change into clothing that was free of metal and/or hard plastic (*e.g.*, buttons, zippers, snaps, etc.) and asked to remove all metal from the body (*e.g.*, jewelry, eyeglasses, hair accessories, etc.). The following scans were performed on each subject: 1) anteroposterior (AP) view of the total body with the subject lying supine; 2) AP view of the lumbar (L1-L4) spine with the subject lying supine with hips and knees supported at a 90º angle; and 3) AP view of the right and left femoral neck with the subject lying supine with thigh internally rotated. Testing was completed according to the manufacturer's instructions and specifications by a certified X-ray technician. Evidence demonstrates that 12 week intervals are sufficient to observe significant changes in BMD in comparable populations (5).

Following the anthropometric measurements, subjects performed an incremental exercise test on an electronically braked cycle ergometer (RacerMate; Velotron Dynafit Pro, Seattle, WA). Subjects began exercising at 50 W with resistance increasing by 50 W every two minutes up to 150 W. Thereafter, resistance increased by 25 W every minute until volitional fatigue. The criterion for achievement of VO_2 max was fulfilled by reaching at least three of the following: 1) a plateau in oxygen consumption for an increase in exercise intensity (< 2.0 mL/kg/min increase), 2) respiratory exchange ratio = 1.05), 3) heart rate = 85% of an age predicted maximum (as determined by 220-subject's age), 4) voluntary cessation of the test by the subject and 5) a rating of perceived exertion (RPE) > 18 on the Borg Scale (6). Expired gasses were measured continuously using a TrueMax 2400 metabolic cart (ParvoMedics, Sandy, UT).

Following 15 minutes of recovery post-VO_2 max testing, those (N=20) with VO_2 max capacity > 50.0 mL/kg/min performed a 40 km TT. The sole purpose of including a pre and post-intervention TT was to provide greater objective evidence (in contrast to self-reported training logs) that subjects maintained sufficient training volume during the 12-week study; since identical procedures were followed during pre and post-testing, potential fatigue from the VO_2 max test was not considered to be a confounding variable on the results. During the TT, a computer display provided subjects with gearing, current and average speed, cadence, grade

and distance. For purposes of standardization, no outside encouragement was offered during any aerobic testing and subjects were not allowed to listen to music. A familiarization trial was not utilized due to the high levels of cycling experience and because TT data were not used as a primary outcome measure. Blood samples were again taken immediately post-exercise.

Methodology: Treatment Assignment — in an effort to maintain homogeneous groups, trained subjects were ultimately stratified based on age, body mass, and BMD measurements. Subjects were either provided 6 g/d of CCC (containing 600 mg calcium carbonate and 400 IU vitamin D3 (1,25-dihydroxyvitamin D_3) (N=10) (KoACT, AIDP, Inc., City of Industry, CA) or an inert compound of maltodextrin with equivalent calcium and vitamin D concentrations (CON; N=10). A FDA-regulated pill maker was utilized to make all pills, which ensured identical appearance between treatments.

The collagen utilized was a type I collagen hydrolysate formed via biosynthesis, and has been shown to favorably affect markers of bone metabolism (10). A 6 g/d dosage and 12 week intervention duration were chosen based off of previous work in post-menopausal women with comparable BMD to the current study population who experienced favorable bone effects with 5 g/d over 10 weeks (17).

The decision to use a control treatment (calcium+vitamin D alone) instead of a true (inert) placebo is justified since all primary dependent variables are unaffected by conscious control or subconscious bias. Subjects in both groups consumed their respective supplements twice daily (four pills in the morning and four pills in the evening) for the next 12 weeks. They returned empty supplement containers and training/compliance logs every two weeks, at which time they were given supplements for the next two weeks.

Methodology: Compliance and Post-testing —Training and supplementation compliance logs were checked every 4 weeks; any subject that fell below six hours per week of training for two consecutive weeks or a total of four weeks during the intervention period was removed from the study. Likewise, any subject that dropped below 80% compliance with their respective supplement was removed from the study.

Following 12 weeks of supplementation, subjects reported to the laboratory following identical preparation procedures to the initial testing session. Subjects were asked to replicate their initial three-day dietary log during the final three days of the intervention, and repeat all measurements and exercise testing performed during the initial laboratory visit.

Statistical Analysis — Descriptive statistics (means ± SD) were performed on demographic, anthropometric, and serum biomarker variables.

A (one-way analysis of variance) ANOVA was used to compare baseline variables between the CON and CCC groups. Effects of the supplementation protocol on BMD (whole body, total hip, lumbar spine), BAP, TRAP5b, and SCL were evaluated by a two-way repeated measures ANOVA (group x time). A Tukey post hoc test was used to compare group values when significance was found, and Pearson product-moment correlations were applied during a secondary analysis (ranges were determined as 0.0-0.3 = weak, 0.3-0.7 = moderate, 0.7-1.0 = strong). Significance was accepted at $p < 0.05$, and all statistical analysis were performed using SPSS version 19 (IBM, Armonk NY).

3. Results

Thirty-nine healthy, non-smoking men, currently riding at least three hours per week, volunteered to participate in this study. Email and telephone screening were used to identify twenty-nine subjects eligible for participation. Following initial screening and VO_2 max testing, twenty subjects had qualified as trained (VO_2 max > 50.0 mL/kg/min). Two subjects were later removed from the study due to failure to maintain training volume and supplement

compliance. The remaining 18 subjects (CCC N=9; CON N=9) completed the entire protocol with 100% compliance and were included in all analyses. See Table 1 for the descriptive characteristics of the trained cyclists who completed the entire study protocol, as well as Figure 1 which outlines the subject selection and rejection process; there were no significant baseline differences ($p > 0.05$) between groups for any analysed variable.

No between-group significant differences were expected for VO_2 max or TT data, as the tests were used solely to qualify the subjects as "trained" and to ensure sustained training volume during the intervention. As hypothesized, no between-group effects were observed; however, multiple significant ($p < 0.05$) time effects occurred in both groups. Performance testing outcomes can be seen in Table 2.

No within or between group differences for whole body BMD or at the individually measured sites were found. Pre- and post-intervention BMD data are presented in Table 3. Significant ($p < 0.05$), positive Pearson moment correlations were found when VO_2 max power (maximum power achieved during the maximal graded exercise test) and whole body BMD were compared (r = 0.549). Moderate, positive correlations also were found between VO_2 max and right and left hip BMD (r = 0.495 and r = 0.522, respectively). VO_2 max power to weight ratio was moderately correlated to lumbar, right and left hip BMD (r = 0.47, r = 0.528 and r = 0.521, respectively).

Biomarkers of bone metabolism results are presented in Table 4. No significant within or between trial effects on BMD were identified in either group. Significant ($p < 0.05$),

Table 2. Means and Standard Deviation for Performance Variables

	CCC (N=9)			CON (N=9)		
Variable	Pre	Post	*d*	Pre	Post	*d*
VO_2 max (mL/kg/min)	58.7 ± 3.5	59.1 ± 4.2	0.10	57.0 ± 4.8	57.8 ± 5.1	0.16
VO_2 max Power (W)	402.8 ± 42.3	397.2 ± 50.7	-0.12	416.7 ± 61.2	405.6 ± 65.9	-0.17
VO_2 max Power/Weight (W/kg)	5.4 ± 0.4	5.3 ± 0.4	-0.25	5.3 ± 0.5	5.2 ± 0.6	-0.18
TT Mean Power (W)	248.9±35.6	261.6±42.5*	0.32	264.0±58.6	267.6±65.3*	0.06
TT Power/Wt (W/kg)	3.3 ± 0.4	3.5 ± 0.4*	0.50	3.3 ± 0.5	3.4 ± 0.5*	0.20
TT Time to Completion (min)	66.9 ± 3.6	65.5 ± 3.9*	-0.37	65.6 ± 4.8	64.6 ± 5.1*	-0.20

Note. CCC = calcium collagen chelate supplementation group; CON = control group; VO_2 max = ma oxygen consumption; TT = time trial; * Significantly different from pretest condition at $p < 0.05$.

Table 3. Means and Standard Deviation for Body Composition and Bone Mineral Density

	CCC (N=9)			CON (N=9)		
Variable	Pre	Post	*d*	Pre	Post	*d*
LBM (kg)	57.6 ± 6.1	57.0 ± 6.3	0.10	59.4 ± 7.2	60.2 ± 7.8	0.10
Body Fat (%)	18.2 ± 4.3	19.4 ± 4.7	0.27	20.6 ± 2.8	18.9 ± 3.2	-0.57
Whole Body	1.120 ± 0.082	1.088 ± 0.066	-0.43	1.113 ± 0.095	1.114 ± 0.098	0.01
Spine (L1-L4) (g/cm^2)	0.929 ± 0.118	0.923 ± 0.119	-0.05	0.916 ± 0.139	0.922 ± 0.136	0.04
Right Hip (g/cm^2)	0.933 ± 0.062	0.940 ± 0.064	0.11	0.871 ± 0.104	0.866 ± 0.101	-0.05
Left Hip (g/cm^2)	0.924 ± 0.054	0.932 ± 0.054	0.15	0.868 ± 0.100	0.865 ± 0.101	-0.03

Note. LBM = lean body mass; CCC = calcium collagen chelate supplementation group; CON = control group

Table 4. Means and Standard Deviation for Resting Biomarkers of Bone Metabolism

	CCC (N=9)			CON (N=9)		
Variable	Pre	Post	*d*	Pre	Post	*d*
BAP (U/L)	31.73 ± 7.63	32.87 ± 5.37	0.17	36.16 ± 7.72	35.61 ± 7.39	-0.07
TRAP5b (U/L)	3.29 ± 0.95	3.45 ± 0.88	0.17	2.97 ± 0.72	2.99 ± 0.67	0.03
BAP/TRAP	9.93 ± 2.42	9.91 ± 2.31	-0.01	12.64 ± 3.35	12.51 ± 3.83	-0.04
SCL (ng/mL)	0.45 ± 0.13	0.45 ± 0.08	0.11	0.44 ± 0.07	0.44 ± 0.06	0.00

Note. CCC = calcium collagen chelate supplementation group; CON = control group; BAP = bone alkaline phosphatase; TRAP5b = tartrate resistant acid phosphatase 5b; SCL = sclerostin.

moderate correlations were found between age and TRAP5b as well as BAP/TRAP5b ratio (r = -0.664 and r = 0.589, respectively). Training years and training hours were also moderately correlated with TRAP5b (r = -0.546 and r = 0.531, respectively). Comparison of VO_2 max to the biomarkers revealed a negative correlation between BAP and BAP/TRAP5b ratio (r = -0.561 and -0.597, respectively).

In regard to the relationships between measures of BMD and the biomarkers of bone metabolism, BAP was significantly (p < 0.05) negatively correlated to right hip BMD (r = -0.660) and approaching a significant (p = 0.059) negative correlation to left hip BMD (r = -0.467). BAP/TRAP5b ratio was significantly negatively correlated to both right and left hip BMD (r = -0.649 and r = -0.646, respectively). No other biomarkers were significantly correlated with BMD at any site.

4. Discussion

The primary findings of this study were that no significant changes occurred in BMD, body composition, or biomarkers of bone metabolism over the course of 12 weeks of training (CCC = 10.4 ± 2.3 h/wk; CON = 10.3 ± 2.5 hr/wk), regardless of CCC or calcium+vitamin D supplementation alone, in competitively trained cyclists. While 12 weeks is a short duration to observe changes in BMD, previous research has demonstrated that significant changes can be seen in equivalent durations with a comparable, albeit slightly smaller, subject pool (5); this suggests that the present study was sufficient in duration to observe significant changes. The use of a non-compromised and therefore more vulnerable to supplementation population (e.g., older adults) is a limitation to the present study. Likewise, very recent evidence indicates that higher doses of collagen derivatives (~ 15 g) may be a more effective dose for influencing collagen synthesis (19), although no data exist to our knowledge supporting the higher dose for bone-specific outcomes.

Nichols et al. (2011) measured BMD in master cyclists (50.7 ± 4.0 yr) who had trained for 20.4 ± 6.7 yr at over 10 hr/wk; they found similar BMDs to our cyclists at the lumbar (0.937 vs. 0.925 g/cm^2, respectively) and hip regions (0.871 g/cm^2 total hip vs. 0.900 right and 0.896 left hip) (22). However, in our trained subjects, correlations revealed a strong positive relationship between weekly training hours and lumbar BMD (r = 0.473), suggesting more cycling activity was beneficial to BMD. This is in contrast to the theory that significant time in a non-weight bearing position contributes to declines in BMD, particularly at the lumbar spine (24, 26).

In order to get a comprehensive view of bone turnover, markers associated with the three cell types found in bone responsible for resorption and formation activities were measured in the present study. BAP and TRAP5b were analysed to measure the activity of osteoblasts responsible for bone formation and the osteoclasts responsible for bone resorption, respectively. SCL was measured to determine the influence of osteocytes, the most prominent cell type found in bone, since they have been shown to

be the most receptive to mechanical stimulation and to mediate bone turnover (32). While vitamin D status was not assessed, the 12-week training period occurred between March and June at an approximate latitude of 30 degrees North and therefore vitamin D concentrations were likely adequate considering the supplementation dose. Few studies have investigated the effects of cycling on biomarkers of bone metabolism, and only one of these studies examined longitudinal changes under regular training conditions (4, 11, 12, 20, 24).

Present results were similar to previous studies regarding BAP which show no change in cyclists (4, 12, 20). Although reference ranges have not been established in healthy men, comparison of baseline BAP reveals that our values are higher than those previously reported but within the normal range established for healthy pre-menopausal women of 14.8 to 38.8 U/L (9). When BAP was compared to measures of BMD, negative relationships were found at the right hip (r = -0.660) and left hip T-score (r = -0.491). Although BAP is often considered a measurement of bone formation activity, it may also act as a marker of whole bone turnover (3).

While most biomarkers of bone resorption are related to the measurement of degraded byproducts of bone resorption, TRAP5b is the only measure of osteoclast number and activity (7). In a study conducted by Lombardi et al. (2012), investigators observed a significant increase in TRAP5b levels over 22 days during a three-week professional cycling race from approximately 2.5 to > 3.5 U/L. Although similar increases in TRAP5b were not observed in the present study, overall values were similar at 2.97 - 3.45 U/L. These values appear to be slightly higher than those measured (2.7 ± 0.4 U/L) in physically active, healthy men of similar age (25). Since reference values for TRAP5b do not exist, it is difficult to determine the clinical significance of these values. However, when TRAP5b was compared to age and training years, negative correlations were found suggesting that older athletes with more years of training had lower levels of TRAP5b (r = -0.546).

Due to the lack of established reference values for the biomarkers of bone metabolism, the relationship between bone formation and bone resorption in the form of BAP/TRAP5b ratio was examined. Higher values for this ratio may represent an environment that favors formation or may simply represent higher levels of bone turnover. When the BAP/TRAP5b ratio was compared to measures of BMD, the only relationships found were negative at the right (r = -0.649) and left (r = -0.646) hips. If both the resorptive and formative activities were elevated, higher BAP/TRAP5b ratio may simply represent increased bone turnover instead of formation, although critics state that the ratio is not an appropriate indices for any interpretation of bone metabolism.

SCL, released almost exclusively by osteoclasts, may be the most sensitive bone marker to changes in mechanical loading (2). Increased levels of SCL have been associated with reductions in osteoblast activity and could result in reduced BMD. Previous research has linked high levels of SCL to increased risk of vertebral fracture (31). This association is likely due to the Wnt pathway inhibition resulting in reduced osteoblast and bone formation activity (30). Although baseline SCL levels of 0.44 - 0.45 ng/mL in our trained cyclists were low when compared to a study in healthy young men (0.81 ng/mL) and older adult men (1.79 ng/mL), SCL has been shown to be elevated in the short-term and potentially reduced in the long term during exposure to non-weight bearing activities (27, 31). Due to the lengthy training history and low BMD of our cyclists, it is possible that they were producing less SCL due to less mature bone (31).

There are several limitations to the present study. While significant changes in BMD have been reported in periods < or = 12 weeks (5, 18), one of these investigations (18) involved strenuous training of military recruits; it is possible that a 12 week intervention was too short to capture changes in DXA scores in our subject population with

non-weight bearing training. Additionally, physical training exclusive of cycling and dietary habits were not analysed during the 12 week period which may have affected outcomes. However, subjects were restricted from training > 2 days per week in any non-cycling activity. Likewise, while it is assumed that the latitude, season, and supplementation of vitamin D ensured adequate protection against clinically low vitamin D levels, neither vitamin D nor calcium blood levels were assessed which may have provided a more complete profile of bone health.

5. Practical Applications.

While cycling training provides a host of beneficial adaptations related to health and body composition, losses in BMD that often accompany high volume training remain a concern among coaches and athletes. Since CCC supplementation appears to have no significant effects on BMD or markers of bone turnover as compared to calcium+vitamin D alone, coaches are encouraged to utilize alternate adjunctive strategies to attenuate potential bone loss in cyclists, such as ensuring nutritional adequacy and the incorporation of traditional resistance training at appropriate intensities.

6. Conclusions

While cycling is beneficial to cardiovascular outcomes, the results of the present study suggest that bone does not respond in the same favorable manner. We found no effect from CCC and/or calcium+vitamin D supplementation regarding increases in BMD, improvements in body composition, or affecting biomarkers of bone metabolism within a 12-week period. While higher weekly training hours were associated with higher baseline levels of TRAP5b, all measured markers of bone metabolism (*i.e.*, BAP, TRAP5b and SCL) remained at similar levels throughout the study. Chronically high levels of bone turnover would result in less mature bone being maintained in the skeleton. Future work investigating structural changes in bone, as well as long-term fracture incidence and prevalence rates in trained cyclists, should be performed to determine whether or not the reduced BMD experienced by cyclists places these athletes at greater risk for fractures.

Supplementary Materials: None

Funding: This research received no external funding.

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflict of interest.

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