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Correlations Between Changes in Undercarboxylated Osteocalcin and Muscle Function in Hypoparathyroidism

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Abstract

Background: Muscles and bones are interconnected. Recent studies suggest that undercarboxylated osteocalcin from bone may affect muscle mass and strength. There are, however, no prospective human data on this relationship.

Methods: We previously treated patients with hypoparathyroidism with intact Parathyroid Hormone (PTH) or placebo in a six-month randomized, placebo-controlled trial and demonstrated a marked increase in undercarboxylated osteocalcin (ucOC) in the PTH-treated group. We therefore investigated if this increase correlated with changes in muscle mass, strength or function. Primarily, the muscle mass using Dual energy X-ray Absorptiometry (DXA) was measured and the maximal voluntary isometric muscle strength at the upper and lower extremities, using dynamometry, was assessed. Furthermore, repeated chair stands test, Timed Up and Go test were performed and postural stability using a stadiometer was assessed. Finally, the relationship between change in ucOC or the ratio of the changes in ucOC and total OC (ucOC%/OC%) and different measures of muscle function were analyzed, using regression analyses.

Results: The findings indicated that ucOC%/OC% was positively and significantly associated with percentage change in max force production during elbow extension ($\beta = 0.28$, $P = 0.034$), however, all other associations were non-significant.

Conclusions: Given the number of statistical tests that were carried out, our one significant finding may represent a false positive. Thus the results do not support the role of ucOC in muscle function in humans with hypoparathyroidism. Our results are inconsistent with previous data from a human cross-sectional study; however, cross-sectional studies, do not allow for inference of causality. The analyses should be repeated in larger, randomized trials including healthy individuals.

Keywords: Clinical Trial, Undercarboxylated Osteocalcin, Muscle Function, Hypoparathyroidism

1. Background

Muscles and bones are interconnected. A cross-sectional study showed that muscle size and strength correlate positively with both bone size and strength (1), and a number of external factors have similar effects on muscle and bone; e.g. high-intensity weight bearing training increases both muscle and bone mass (2), chronic bedrest reduces both muscle and bone mass (3) and both muscle and bone mass decrease with age (4, 5). Muscles can also affect bones directly. The Mechanostat model states that bone adapts by increasing its mass when muscle contraction deforms bone (6). The deformation of bone is believed to alter the flow in the Haversian canals and bone marrow, which elicits a mechanical stimulation of the osteocytes that again signals to the osteoblasts (7). One of the proteins responsible for mechanotransduction is the gap junction connexin 43 (8). A recent study showed that deletion of the connexin 43 gene in mice bone cells decreased bone mass, and surprisingly also muscle mass, suggesting a feed-back loop. The reduction in muscle mass was accompanied by a reduction in mRNA for Osteocalcin (OC) in bone. Moreover, the phenotype could be rescued by infusion of Undercarboxylated Osteocalcin (ucOC) (9), suggesting that a bone-derived factor can affect muscles. In accordance with this, a human cross-sectional study demonstrated a correlation between ucOC and muscle strength (10). There are, however, no longitudinal human studies investigating the effect of changes in ucOC on muscle mass and strength.

2. Objectives

We previously conducted a randomized, prospective trial in which patients with hypoparathyroidism were randomly assigned to treatment with either intact Parathyroid Hormone (PTH) or placebo (11). The treatment caused a dramatic increase in ucOC of more than 1000% (12). We

therefore tested the hypothesis that this increase affected muscle function.

3. Methods

3.1. Study Population

The study population has been described in our previous study (11). In summary, we randomly assigned 62 patients with hypoparathyroidism to double-blinded treatment with either 100 μg PTH (1-84) (Preotact®, Nycomed, Denmark) or similar placebo for 24 weeks in addition to standard care with calcium and activated vitamin D (clinical trial registration number: NCT00730210). Informed consent was obtained from each patient included in the study and the study protocol conformed to the ethical guidelines of the 1975 declaration of Helsinki. The study was approved by The Danish Data protection agency, the ethical committee of central Denmark (No. M20080040), and the Danish national board of health. In the present analysis, we only included the 58 patients, who completed the trial (30 in the placebo group and 28 in the PTH group).

3.2. Measurements

We measured plasma-ionized calcium at baseline and on weeks 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24, and plasma phosphate and 25-hydroxyvitamin D at baseline and on week 24 using standard laboratory methods. Osteocalcin was measured using standard techniques at the Department of clinical biochemistry, Aarhus University hospital and we determined the plasma levels of ucOC by the enzyme linked immunosorbent assay (ELISA) (Takara Bio Inc., Shiga, Japan, intra-assay CV < 6.66%). We performed all analyses in batches. We measured muscle mass in terms of lean tissue mass by dual-energy X-ray absorptiometry at baseline and after 24 weeks (Hologic Discovery, Hologic Inc, Waltham, MA, USA) and we assessed the maximal voluntary isometric muscle strength both at the upper- and lower extremities on the dominant side with an adjustable dynamometer chair (Good Strength TM, Meitur Ltd, Finland), as previously described (13). Maximal strength was measured in Newton (N) and maximal force production in Newton/second (N/s). We also performed the repeated chair stands (RCS) test measuring the time (s) for 10 consecutive chair stands without the use of hands and the timed up and go test (TUG), i.e. time taken to stand up from a chair, walk 3 m, turn around and walk back to the chair. We assessed postural stability with the use of a stadiometer (Good Balance Platform SystemTM, Metitur Ltd., Finland), as previously described in details (13). The stadiometer records length (mm) and speed (mm/s) of the sway in medio-lateral and anterior-posterior direction. The data were given as velocity moment (VM, mm^2/s), which is a calculated product of total length of sway in both directions per second. The reliability coefficient was 0.53 (14). We measured postural stability under four different conditions: 1) normal standing with eyes open; 2) normal standing with eyes closed; 3) semi-tandem, and 4) tandem standing (13). We adjusted for differences in body height by calculating scaled velocity moment (SVM) = $(VM/(\text{height in cm})^2) \times 180^2$ (15).

3.3. Statistics

The primary efficacy measure of the initial randomized placebo-controlled study was maximal voluntary extension with the knee in a 60° degree angle. According to our sample size calculations, 30 subjects would be needed in each study group to be able to demonstrate a 10% improvement in maximal voluntary muscle contraction (measured as maximal force, SD = 13.5%) at 5% significance level, with a statistical power of 80% based on an independent samples t-test.

The relationship between percentage change in ucOC or the ratio of the percentage changes in ucOC and total OC (ucOC%/OC%) and percentage change in the measures of muscle function described above, was analyzed, using linear regression analyses. The dependent variables were the measures of muscle function and the predictors were percentage change in ucOC or ucOC%/OC%. We adjusted the results for numbers of episodes of hypercalcemia (defined as plasma ionized calcium > 1.32 mmol/L), documented for the individual patient between baseline and 24th week measurement, for the change in plasma levels of 25OHD and phosphate between baseline and week 24, or for the coefficient of variation (CV%) of ionized plasma calcium levels in individual patients, according to plasma calcium levels measured at each visit during the trial in separate analyses. The analyses were conducted for the entire population and in treatment and placebo groups, separately. We evaluated differences in baseline characteristics and changes in ucOC and OC between groups using independent samples t-test for continuous variables and Fisher's exact test for categorical variables. We log-transformed data not normally distributed. Level of significance was 0.05.

4. Results

The baseline characteristics are shown in Table 1. There were no differences regarding age, gender, etiology of hypoparathyroidism, or body composition (Table 1). Baseline results regarding muscle function, OC and ucOC have been published previously (12, 13). The treatment and placebo groups were well balanced with no significant differences in ucOC, OC or any measure of muscle function (12, 13).

Table 1. Baseline Characteristics and Percentage Change in Total Osteocalcin and Undercarboxylated Osteocalcin of the Placebo and Parathyroid Hormone Treated Groups[a,b]

Variable	Placebo (n = 30)	PTH (n = 28)	P Value
Gender (male/female)	4/26	4/24	1.00
Etiology (idiopathic/post-surgical)	2/28	1/27	1.00
Age, y	50.5 ± 11.1	53.8 ± 11.3	0.25
Body weight, kg	80.5 ± 17.9	85.8 ± 19.8	0.27
Body mass index, kg/m^2	28.1 ± 6.1	29.5 ± 5.9	0.36
P-ucOC, μg/L	2.37 (1.54 - 3-64)	3.57 (2.54 - 5.05)	0.15
%-change ucOC	69.3 ± 79.4	1185.0 ± 814.4	< 0.001

Abbreviations: OC, osteocalcin; ucOC, undercarboxylated osteocalcin.
[a]Values are expressed as mean percentage change ± standard deviation (SD) except for P-ucOC where data are median percentage change with CI as statistics have been done on log-transformed data.
[b]P values refer to independent samples t-test.

In response to treatment, ucOC increased markedly by 1185.0 ± 814.4% (mean ± SD) in the treatment group and by 69.3 ± 79.4% in the placebo group (P < 10 - 50, Table 1). In the entire study population, ucOC increased by 428.4 ± 448.3% (P = 10 - 6, data not shown).

During follow-up, ucOC%/OC% was positively and significantly associated with percentage change in max force production during elbow extension (β = 0.28, P = 0.034, Table 2). No other associations were demonstrated (Table 2). Adjustment for numbers of episodes of hypercalcemia, for the CV percentage of ionized plasma calcium, or for percentage change in plasma levels of 25OHD or phosphate did not change the results (data not shown). When conducting the analyses in the placebo or PTH-treated groups separately, with or without adjustments, there were no significant associations (data not shown).

5. Discussion

In the present study, the associations between percent changes in ucOC or ucOC%/OC% and a number of measurements of muscle function, as a secondary analysis from a randomized, double-blinded, placebo-controlled study in patients with hypoparathyroidism, were investigated. It was found that the ucOC%/OC% was positively and significantly associated with max force production during elbow extension (β = 0.28, P = 0.034) whereas none of the other muscle function tests were associated with percent change in ucOC or ucOC%/OC%.

The findings were in line with the results of a study on mice, in which a reduced muscle mass phenotype could be rescued by infusion of ucOC (9). However, given the number of end-points tested in the present study and the fact that the association with elbow extension was the only statistically significant association demonstrated, we cannot exclude that this was an incidental finding.

To the best of our knowledge, only one human study has previously investigated ucOC in relation to muscle function. In contrast to our findings, Levinger et al. (10) found that the ratio of ucOC/total OC was positively and significantly associated with muscle strength at the hip and thigh in 90 females aged 70 years or above. Although the sample size was larger and the methods used to measure muscle strength differed from our study, it should be emphasized that the study by Levinger et al. (10) used a cross-sectional design, which does not allow for causal conclusions.

Muscle strength and function is affected by a number of additional factors including plasma levels of calcium and 25OHD. Thus, previous studies suggest that hypocalcemia may cause myopathy (16, 17), while hypercalcemia in hyperparathyroidism impairs gait speed (18). We have previously reported that fluctuations in plasma calcium levels occurred, which occasionally resulted in mild hypercalcemia among patients of the therapy group with intact PTH (11). However, adjusting our results for individual CVs in plasma calcium levels during the study or number of episodes with hypercalcemia, did not change the results. In addition, plasma 25OHD levels are known to affect muscle function (19) but adjustment for percentage changes in plasma 25OHD levels in the present study did not change the results either.

Strengths of the present study include a follow-up period suitable for demonstrating a change in muscle function, a marked increase of more than 1000% in ucOC as well as the fact that muscle strength was the primary endpoint of the study.

Limitations were as follows; most of the end-points investigated here were secondary end-points, which the study may be under-powered to evaluate properly thus inflating the risk of false negative results; the highly selected population that already had musculoskeletal problems; and the fact that all participants in the study were ambulatory and the results might have been different if the patients had severe myopathy at baseline.

In conclusion, we investigated the associations between changes in ucOC and muscle function in patients with hypoparathyroidism in a prospective study. Our data do not support a role for ucOC with regards to muscle strength, muscle mass, or postural stability in humans, however, randomized controlled studies including healthy individuals should be conducted.

Table 2. The Relationship Between Percentage Change in Undercarboxylated Osteocalcin (ucOC) or the Ratio Between Percentage Change of Undercarboxylated Osteocalcin and Total Osteocalcin (OC) and Percentage Changes in Different Measures of Muscle Function in the Entire Study Population[a]

Measure of Muscle Function	B Value % - Change in ucOC	P Value % - Change in ucOC	B Value Change in ucOC%/OC%	P Value Change in ucOC%/OC%
Elbow extension				
Max force, N	-0.21	0.12	0.04	0.79
Max force production, N/s	-0.03	0.81	0.28[b]	0.03[b]
Elbow flexion				
Max force, N	-0.10	0.45	-0.08	0.55
Max force production, N/s	-0.06	0.67	0.20	0.13
Hand grip				
Max force, N	0.13	0.34	0.06	0.65
Max force production, N/s	-0.15	0.26	0.18	0.18
Knee extension 60°				
Max force, N	-0.03	0.80	0.04	0.76
Max force production, N/s	-0.16	0.22	0.12	0.38
Knee flexion 60°				
Max force, N	-0.10	0.48	0.02	0.91
Max force production, N/s	0.07	0.62	-0.02	0.88
Knee extension 90°				
Max force, N	-0.04	0.75	-0.05	0.71
Max force production, N/s	0.19	0.16	0.00	0.99
Knee flexion 90°				
Max force, N	-0.06	0.66	-0.03	0.80
Max force production, N/s	-0.05	0.70	0.23	0.08
Normal standing eyes open				
Velocity moment, m^2/s	-0.11	0.41	-0.00	0.98
Normal standing eyes closed				
Velocity moment, m^2/s	0.15	0.26	0.00	0.99
Semi tandem standing				
Velocity moment, m^2/s	-0.09	0.49	-0.09	0.51
Tandem standing				
Velocity moment, m^2/s	-0.08	0.57	0.21	0.12
Total lean mass, g	-0.15	0.27	-0.05	0.70
Repeated chair stands, s	0.13	0.34	0.09	0.52
Timed up and go, s	0.08	0.57	0.01	0.95

[a] Values are expressed as β values from unadjusted linear regression with corresponding P value.
[b] Statistically significant correlation and P value < 0.05.

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Footnote

Authors' Contribution: Study concept and design: Torben Harslof, Tanja Sikjaer, Bente L. Langdahl, Mosekilde and Lars Rejnmark; acquisition of data: Torben Harslof and Tanja Sikjaer; analysis and interpretation of data: Torben Harslof, Tanja Sikjaer, Mosekilde, Bente L. Langdahl and Lars Rejnmark; drafting of the manuscript: Torben Harslof; critical revision of the manuscript for important intellectual content: Torben Harslof, Tanja Sikjaer, Moskilde, Bente L. Langdahl and Lars Rejnmark; statistical analysis: Torben Harslof, Tanja Sikjaer and Lars Rejnmark; administrative, technical and material support: Mosekilde, Bente L. Langdahl and Lars Rejnmark; study supervision: Mosekilde, Bente L. Langdahl and Lars Rejnmark.

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