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Serum Markers of Bone Turnover and Angiogenesis in Bisphosphonate-related Osteonecrosis of the Jaw Patients Following Discontinuation of Long-term Intravenous Bisphosphonate Therapy

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Abstract

Purpose—To analyze serum markers of bone turnover, angiogenesis, endocrine function, and inflammation in bisphosphonate-related osteonecrosis of the jaw (BRONJ) patients who discontinued long-term intravenous bisphosphonate (BP) therapy.

Patients and Methods—Serum samples were obtained from 25 BRONJ patients who had discontinued long-term intravenous BP therapy for an average of 11.4±8.7 months and 48 non-

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BRONJ controls who continued receiving intravenous BP therapy. Samples were analyzed for total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OCN), C-telopeptide (CTX), vascular-endothelial growth factor (VEGF), triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), 25-hydroxyvitamin D, and C-reactive protein (CRP).

Results—The mean number of BP infusions was significantly higher in BRONJ subjects compared with controls (38.4 ± 26.3 infusions vs 18.8 ± 7.2 , $p < 0.0001$); however, the duration of BP therapy was not significantly different between the groups ($p = 0.23$). Overall, there were no significant differences in any of the markers between BRONJ subjects and controls (all p values 0.16). In a subgroup analysis that matched BRONJ subjects and controls according to mean age and BP infusions (BRONJ, $n = 10$ and controls, $n = 48$), $\log_{10}$ VEGF (2.9 ± 0.4 vs 2.4 ± 0.4 , $p = < 0.001$) and CRP (34 ± 26 vs 13 ± 8 , $p = < 0.01$) levels were significantly higher in BRONJ subjects compared with controls. Within BRONJ subjects, none of the serum markers were correlated with duration of BP discontinuation.

Conclusions—Bone turnover and endocrine markers in BRONJ subjects who discontinue long-term intravenous BP therapy are similar to non-BRONJ controls receiving intravenous BP therapy. However, angiogenesis and inflammation markers are higher in BRONJ subjects who discontinue long-term intravenous BP therapy. The prolonged skeletal half-life of BPs may suppress bone turnover markers in BRONJ subjects for several years following discontinuation of intravenous BP therapy, suggesting an extended effect on bone homeostasis.

Introduction

Bisphosphonates (BP), which are potent inhibitors of osteoclast-mediated bone resorption, are associated with bisphosphonate-related osteonecrosis of the jaw (BRONJ).^{1,2} To date, the etiology and pathogenesis of BRONJ development remains unknown. It is hypothesized that long-term BP therapy may impair bone turnover and angiogenesis processes, predisposing an individual to BRONJ development.³

Previous reports suggest that over-suppression of osteoclasts by long-term BP therapy can affect osteoblast function, thereby impairing bone renewal.^{3,4} In the absence of effective bone remodeling, events such as tooth extractions and dental infections might result in tissue death, vascular loss, and eventually osteonecrosis. BP's alternative pharmacological actions include angiogenesis inhibition^{5,6} and immune modulation.⁷ Treatment with pamidronate or zoledronate has been reported to reduce vascular endothelial growth factor (VEGF) levels^{5,6} and bone blood flow in rats.⁸ These findings suggest that BP's anti-angiogenic activity in jawbones could induce avascular necrosis and impair tissue repair.

Several cross-sectional studies have attempted to investigate the predictive value of bone turnover markers (e.g., C-terminal telopeptide (CTX), N-terminal telopeptide (NTX), osteocalcin, alkaline phosphatase) and angiogenesis markers (e.g., vascular endothelial growth factor (VEGF)) in BRONJ development.^{9–12} Currently, no scientific evidence exists to support the use of these serum markers in assessing BRONJ risk.¹³ Based on observational findings and expert opinion, some reports have suggested drug holiday as an additional approach to improve outcomes for patients in need of invasive dental procedures.^{9,14,15} It is believed that discontinuing BP therapy might mitigate the risk of

developing BRONJ or facilitate BRONJ healing by improving impaired bone remodeling process. However, no scientific evidence exists to support the discontinuation of BP therapy and little is known about the bone turnover and angiogenesis markers in BRONJ patients following discontinuation of long-term BP therapy.

This study aims to serologically characterize markers of bone turnover, angiogenesis, endocrine function, and inflammation in BRONJ patients who discontinued long-term intravenous BP therapy.

Patients and Methods

Subjects

The University of Minnesota Institutional Review Board approved this case-control study and written informed consent was obtained from all subjects. Cancer patients 30 years of age or older, who had received intravenous BP therapy for cancer management were included in this study. Patients with a history of either radiation therapy to the head and neck region or neoplasms (including metastasis) involving the head and neck region were excluded. Patients were recruited from oncology centers in Twin Cities area and from the University of Minnesota School of Dentistry from July 2007 through July 2012. Eligible cases were defined as patients with a confirmed diagnosis of BRONJ who had discontinued receiving long-term BP therapy. The American Association of Oral and Maxillofacial Surgeons criteria¹⁴ was used to diagnose BRONJ: “exposed bone in the maxillofacial area occurring in the absence of head and neck irradiation and showing no evidence of healing for at least 8 weeks after identification in patients treated with BP therapy”. The control group consisted of non-BRONJ cancer patients who had received at least 10 intravenous BP infusions and continued to receive BP therapy at enrollment.

Serum was isolated from venous blood and stored at -80°C for future analysis.

Laboratory Procedures

Serum samples were assayed for bone-specific alkaline phosphatase (BALP) (Immunodiagnostic Systems Inc., Scottsdale, AZ); c-telopeptide (CTX) (Immunodiagnostic Systems Inc., Scottsdale, AZ); vascular-endothelial growth factor (VEGF) (Quantikine ELISA Kit, R&D Systems, Minneapolis, MN); and total alkaline phosphatase (ALP); osteocalcin (OCN); triiodothyronine (T3); thyroxine (T4); thyroid-stimulating hormone (TSH); 25-hydroxyvitamin D; and C-reactive protein (CRP), all from Fairview Health Services, Minneapolis, MN.

Statistical Analysis

Characteristics of cases and controls were compared using Fisher’s exact test or Pearson’s chi-squared test for categorical variables and using two-sample t-tests for continuous measures. For VEGF analysis, Log_{10} transformation removed most of the skewness and kurtosis, leaving a nearly Gaussian distribution verified by normal probability plots. A p-value of <0.05 was considered statistically significant.

Results

Patient Characteristics

Table 1 lists select patient characteristics. There were no significant differences in age ($p=0.50$), sex ($p=0.60$), or race ($p=1.00$) between BRONJ and control subjects. Both BRONJ and control subjects had received intravenous BP therapy for a longer period (mean, 45.1 ± 29.7 months versus 37.7 ± 21.9 months, $p=0.23$). The mean number of BP infusions was significantly higher in BRONJ subjects compared to controls (38.4 ± 26.3 infusions versus 18.8 ± 7.2 ; $p<0.0001$). Most subjects had received BP therapy for metastatic breast cancer management (13 BRONJ and 27 controls).

At the time of sampling, BRONJ subjects had discontinued intravenous BP therapy for a mean period of 11.4 months (range, 2 to 30 months) following initial BRONJ diagnosis. All controls, on the other hand, were receiving intravenous BP therapy for cancer management at the time of sampling.

Laboratory Results

Mean serum levels of markers of bone turnover, angiogenesis, endocrine function, and inflammation are shown in Table 2. There were no significant differences in mean values of ALP, BALP, osteocalcin, CTX, $\log_{10}$ VEGF, T3, T4, TSH, 25-hydroxyvitamin D, and CRP between BRONJ and control subjects.

Despite our best efforts to match cases and controls for BP infusions, BRONJ subjects had significantly higher BP infusions than controls. To address this imbalance, we compared serum markers between a subgroup of BRONJ subjects ($n=10$) and controls ($n=48$) whom we closely matched for mean age (64 years) and number of BP infusions (mean, 19 infusions). Within this well-matched subgroup, and consistent with the unmatched results, we found no significant differences in bone turnover or endocrine markers between BRONJ subjects and controls (Table 3). However, $\log_{10}$ VEGF (2.92 ± 0.38 vs 2.41 ± 0.35 , $p<0.001$) and CRP (34 ± 26 vs 13 ± 8 , $p<0.01$) levels were significantly higher in BRONJ subjects compared with controls. Of these 10 BRONJ subjects, five had discontinued BP for 6 months or less (range, 2 to 6 months) and five had discontinued BP more than 6 months (range, 7 to 22 months). Both in this subset of BRONJ subjects and the entire BRONJ group, none of the serum markers were correlated with duration of intravenous BP discontinuation.

Discussion

It is unknown whether discontinuing intravenous BP therapy affects markers of bone turnover and angiogenesis in BRONJ patients. The present study evaluated serum markers of bone turnover, angiogenesis, endocrine function, and inflammation and found no significant differences in bone turnover and endocrine markers in BRONJ subjects who discontinued long-term intravenous BP therapy and non-BRONJ controls who continued receiving intravenous BP therapy. However, when closely matched for age and number of BP infusions, angiogenesis and inflammation markers appeared to differ between BRONJ subjects who discontinued BP therapy and controls.

Bone resorption markers

Among bone resorption markers, CTX is a highly sensitive indicator of bone resorption and is easily measured by ELISA.^{16,17} Several reports suggest a relationship between suppressed levels of serum CTX and the risk of developing BRONJ.^{9,10,18,19} In one of the earliest reports, Marx et al. retrospectively evaluated 30 BRONJ patients receiving oral BP and reported that serum values of <100 pg/mL represented a high risk for BRONJ development, values between 100 pg/mL and 150 pg/mL represented moderate risk, and values >150 pg/mL represented minimal risk.⁹ Marx's study, however, did not include a control group and only 17 out of 30 patients had CTX data. In a more recent prospective study, Hutcheson et al. evaluated CTX values in 905 osteoporosis patients on oral BP therapy and reported that CTX value of <150 pg/mL at the time of tooth extraction was associated with 3-fold greater risk of BRONJ.¹⁹ In their study, 4 out of 181 patients with CTX value of <150 pg/mL at the time of extraction developed BRONJ, while none of the patients with CTX value of >150 pg/mL developed BRONJ. Similarly, a clinical study by Lazarovici et al. evaluating both osteoporosis and cancer patients found a CTX value of <150 pg/mL was significantly associated with BRONJ development (odds ratio=5.27, p=0.004).¹⁰ In contrast to these findings, Bagan et al. found no relationship between serum CTX and the risk of developing BRONJ in patients receiving both oral²⁰ and intravenous BP's.²¹ Kunchur et al. evaluated patients on oral BP (n=215) and intravenous BP (n=7) scheduled for tooth extractions and reported that CTX test did not predict the development of BRONJ; however, a value of less than 150 to 200 pg/mL suggested patients in the "risk zone".²² Given these conflicting results, several groups, including the American Society for Bone and Mineral Research Task Force on BRONJ and American Dental Association, have not endorsed the CTX test as a predictor for BRONJ development.^{13,23,24}

Long-term clinical studies show that bone resorption markers remain suppressed for several years following discontinuation of intravenous BP therapy.^{25–29} A randomized clinical trial in postmenopausal women noted that a single dose of 5 mg intravenous zoledronate stably decreased bone turnover and increased bone mineral density that lasted for at least 5 years.²⁶ Throughout the 5-year follow-up period, the mean level of β -CTX remained 48% lower in the zoledronate group compared with placebo group. The HORIZON-PFT trial noted a non-significant increase in β -CTX levels in osteoporosis women who discontinued 6 years of intravenous zoledronate treatment compared to those who continued zoledronate for 9 years.²⁷ Nonetheless, β -CTX levels never reached the pre-treatment levels in both the groups. In a recent clinical trial, Patel et al. evaluated bone remodeling markers in multiple myeloma patients under remission, who had previously received 8 to 12 doses of intravenous pamidronate or zoledronate.²⁵ The authors found that NTX levels remained suppressed during the 6-month follow-up period. The ZOTEC study assessing the effect of zoledronate on bone turnover makers in prostate and breast cancer patients found that intravenous zoledronate significantly decreased CTX levels during the course of therapy (4 months), which remained suppressed at 12 months follow-up.²⁸ In the single-arm SubDue study in metastatic breast cancer patients, Simmons et al. noted that 73% of patients who had received a single dose of intravenous zoledronate had continued suppression of serum CTX at 12 weeks.²⁹

In the present study, there was no significant difference in CTX values between BRONJ subjects who discontinued long-term intravenous BP therapy and controls who continued receiving intravenous BP therapy. A plausible explanation for this insignificant difference is that following discontinuation of intravenous BP therapy, CTX levels may remain suppressed and never reach the pre-BP exposure levels in BRONJ subjects, which is consistent with the previous intravenous BP efficacy studies.^{25–28} Similar to the intravenous BP studies, high-quality, randomized, double-blind, fracture intervention clinical trials have noted bone resorption markers remain suppressed in osteoporosis patients up to 10 years following discontinuation of long-term oral BP therapy.^{30–32} In contrast, few retrospective chart review studies have observed an increase in bone turnover markers following discontinuation of oral BP therapy.^{33,34} Currently, it is unknown if discontinuation of long-term oral and intravenous BP therapy have different effects on bone turnover markers. Once incorporated into bones, BPs can exert anti-osteoclastic activity long after discontinuation due to their prolonged skeletal half-life, which is estimated to be around 10 years.³⁵

Bone formation markers

Previous studies have also evaluated bone formation markers in BRONJ development.^{10,11,36} In a prospective study investigating the predictive value of BALP, CTX, and parathyroid hormone in BRONJ development, Lazarovici et al. noted that among patients receiving intravenous BP therapy for cancer management, the mean BALP level did not differ significantly between BRONJ and non-BRONJ patients (22.32 versus 20.78 pg/mL, $p > 0.05$).¹⁰ A large retrospective study from Memorial Sloan-Kettering Cancer Center found no trends in BALP and NTX values in bone metastasis patients before the diagnosis of ONJ.³⁷ The median NTX level increased from baseline value of 8.0 to 10.4 nmol/L at the time of BRONJ diagnosis, while the median BALP level remained unchanged at 18.0 U/L. In contrast to these findings, an exploratory gene expression-profiling study in multiple myeloma patients receiving intravenous BP noted that genes involved in osteoclast and osteoblast signaling, activation, or differentiation were significantly down-regulated in BRONJ patients compared to non-BRONJ patients ($p < 0.05$).³⁸ Consistent with the gene expression results, serum bone formation and bone resorption markers were decreased in BRONJ patients, indicating impaired bone homeostasis in BRONJ subjects at both the transcriptional and protein level.

Previous clinical studies indicate that following discontinuation of intravenous BP therapy, the mean values of bone formation markers remain suppressed below baseline levels.^{25,27,31,39} The HORIZON-PFT trial noted a modest increase in PINP and BALP level following discontinuation of intravenous zoledronate therapy for 3 years, however, the values never reached the pre-treatment level.²⁷ In HIV-infected men, two annual doses of 4 mg intravenous zoledronate showed sustained and stable suppression of osteocalcin and CTX that lasted at least 5 years.³⁹ The ZOTEC study assessing cancer patients found that 4 months of intravenous zoledronate therapy significantly decreased PINP levels, which remained suppressed at 12 months follow-up.²⁸ In contrast to these findings, Patel et al. noted that BALP ($p = 0.003$), osteoprotegerin ($p = 0.19$) and osteopontin ($p = 0.27$) modestly increased after a single dose of zoledronate in multiple myeloma patients, who were previously exposed to 8 to 12 doses of pamidronate or zoledronate.²⁵

In the present study, we found no significant difference in mean ALP, BALP, and osteocalcin levels between BRONJ subjects and controls. These results could be explained by the fact that the bone resorption and bone formation markers remain suppressed for several years after discontinuation of intravenous BP therapy,^{27,31,39} nullifying the difference between patients who continue or discontinue BP therapy.

Angiogenesis markers

It has been hypothesized that angiogenesis suppression by BPs might play an important role in BRONJ development. A clinical study by Vincenzi et al. reported that anti-angiogenic properties of BP are directly linked to BRONJ pathogenesis.¹² Six of the 81 cancer patients who developed BRONJ in Vincenzi et al. study (after a median number of 9 intravenous BP infusions) showed a significant decrease in circulating VEGF levels at day 7 and day 21 after the first BP administration. However, it is unclear whether BRONJ patients in this study had concurrently received other anti-angiogenic agents for cancer treatment. An immunohistochemical study by Wehrhan et al. in 20 BRONJ patients exposed to intravenous BP reported that angiogenesis, but not vascularization, was impaired in mucoperiosteal tissues adjacent to BRONJ lesions.⁴⁰ The authors indicate that tissue regeneration in BRONJ patients may be impaired due to subdued vessel remodeling and new vessel formation.⁴⁰ Furthermore, a possible haplotype effect of VEGF polymorphism has been reported in female BRONJ patients with a history of intravenous BP use.⁴¹

Moreover, cases of exposed jawbone very similar to BRONJ have been reported in patients treated with sunitinib and bevacizumab.^{42–46} Both sunitinib and bevacizumab are antiangiogenic agents used in cancer treatment. It is estimated that BRONJ frequency is higher in patients receiving combined BP and antiangiogenic treatment compared with patients receiving BP therapy alone.⁴²

In the present study, when serum samples were closely matched according to age and BP infusions, VEGF levels were significantly higher in BRONJ subjects compared with controls. The effect of discontinuation of long-term intravenous BP therapy on VEGF levels is unknown. In the present study, there was no wide difference in chemotherapeutic regimen between BRONJ subjects and controls including antiangiogenic agents.

Other markers

Thyroid hormone is essential for skeletal development and plays an important role in maintaining optimal bone strength. Our previous retrospective chart review study indicated that hypothyroidism is an important risk factor for BRONJ development (HR=3.59; 95% CI=1.31–9.83; p=0.013).⁴⁷ In the present study, we found no significant differences in T3, T4, and TSH levels between BRONJ subjects and controls. Furthermore, there was no significant difference in total 25-hydroxyvitamin D levels between BRONJ subjects and controls. In the subgroup analyses matched according to age and BP infusions, the CRP levels were significantly higher in BRONJ subjects compared with controls. It is unknown if the increased CRP level is related to cancer progression or osteonecrosis seen in BRONJ subjects.

In conclusion, serum makers of bone turnover and endocrine function in BRONJ subjects who discontinue long-term intravenous BP therapy are similar to non-BRONJ controls receiving intravenous BP therapy. However, markers of angiogenesis and inflammation appear to differ in BRONJ subjects who discontinue long-term intravenous BP therapy compared with controls. In this study, serum markers in both BRONJ and control subjects were within the reference range for all tests. It is unknown if prolonged half-life of BPs have different effects on serum markers of bone turnover, angiogenesis, endocrine function and inflammation following discontinuation of intravenous BP therapy. The cross-sectional nature of this study limited us from measuring the baselines values of serum markers at the start of BP therapy. Therefore, it is unknown whether serum markers in BRONJ subjects ever return to pre-BP exposure levels following discontinuation of intravenous BP therapy. In addition, it is unclear if discontinuation of long-term oral and intravenous BP therapy have different effects on serum markers of bone turnover and angiogenesis.

Many of the subjects in both BRONJ and control groups had progressive metastatic skeletal disease that could have affected the levels of bone turnover markers, which integrate remodeling activity for the entire skeleton. Furthermore, inflammation and angiogenesis markers are expected to be elevated in BRONJ patients given their history of chronic infection. Measuring VEGF and inflammatory markers at the time of BRONJ development or BP cessation is critical in assessing BP's anti-angiogenic role in BRONJ development. This study primarily focused on intravenous BP. Determining the effects of discontinuation of other antiresorptive medications could provide critical information regarding the role of impaired bone turnover and angiogenesis in ONJ development and its management. Also, this study compared BRONJ subjects who discontinued BP therapy with non-BRONJ controls receiving BP therapy. As a standard practice, patients are generally advised to discontinue BP treatment as soon as they are diagnosed with BRONJ; therefore it is difficult to enroll BRONJ subjects who continue to receive BP therapy. Nonetheless, results from this study provide insight into the effects of intravenous BP discontinuation on serum markers and questions the merit of drug holiday recommended to mitigate the risk of developing BRONJ. Future prospective studies should broadly evaluate the effects of discontinuation of antiresorptive medications on markers of bone turnover and angiogenesis in ONJ patients.

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Table 1Baseline Patient Characteristics (N=73)[#]

Characteristics	BRONJ (N=25)	Non-BRONJ (N=48)	P*
Age – mean±sd, in years	66.3±10.3	64.4±11.7	0.50
Sex – no. (%)			0.60
Female	16 (64%)	34 (71%)	
Race – no. (%)			1.00
White	24 (96%)	46 (96%)	
Black	1 (4%)	2 (4%)	
Malignant disease – no. (%)			0.02
Breast cancer	13 (52%)	27 (56%)	
Multiple myeloma	4 (16%)	15 (31%)	
Prostate cancer	2 (8%)	6 (13%)	
Lung cancer	2 (8%)	0 (0%)	
Renal cell carcinoma	3 (12%)	0 (0%)	
Others	1 (4%)	0 (0%)	
Pre-existing medical conditions – no. (%)			
Diabetes mellitus	1 (4%)	5 (10%)	0.66
Hypertension	11 (44%)	26 (54%)	0.47
Hypercholesterolemia/Hyperlipidemia	11 (44%)	15 (33%)	0.45
Hypothyroidism	7 (28%)	10 (21%)	0.56
Type of bisphosphonates – no. (%)			0.71
Zoledronate	16 (64%)	32 (67%)	
Pamidronate	2 (8%)	6 (13%)	
Pamidronate + Zoledronate	7 (28%)	10 (21%)	
Bisphosphonate infusions – mean±sd			
All cancers	38.4±26.3	18.8±7.2	<0.0001 [‡]
Duration of bisphosphonate exposure – mean±sd, in months			
All cancers	45.1±29.7	37.7±21.9	0.23

* P-values were calculated from a Fisher's exact test or Pearson's chi-squared test for categorical variables and two-sample t-tests for continuous variables.

[‡]P = 0.0011 allowing unequal variances in the two groups.

[#]Table adapted from Thumbigere-Math et al., "Periodontal disease as a risk factor for bisphosphonate-related osteonecrosis of the jaw".⁴⁸ Adapted with permission from the American Academy of Periodontology.

Table 2

Serum Biochemical Markers

Biochemical markers	BRONJ (N=25) [*]	Non-BRONJ (N=48)	Reference range	P [‡]
Bone formation				
Total alkaline phosphatase	98±52	85±76	40–150 U/L	0.45
Bone-specific alkaline phosphatase	13±7	14±8	6.5–22 µg/L	0.58
Osteocalcin	12±4	14±10	11–50 ng/mL	0.25
Bone resorption				
C-telopeptide	228±121	222±119	50–580 pg/mL	0.83
Angiogenesis				
Log10 Vascular-endothelial growth factor	2.42±0.45	2.30±0.34	1.78–2.85 pg/mL	0.23
Endocrine				
Triiodothyronine	118±29	110±30	60–180 ng/dL	0.28
Thyroxine	10±2	9±2	5–11 µg/dL	0.70
Thyroid-stimulating hormone	2±1	2±2	0.4–3.0 µIU/mL	0.21
25-hydroxyvitamin D	38±6	31±10	30–75 µg/L	0.16
Inflammation				
C-reactive protein	20±19	13±8	0–10 mg/L	0.20

^{*} Data were missing for one BRONJ patient.

[‡] P-values were calculated from two-sample t-tests.

Table 3

Serum Biochemical Markers Matched According to Mean Age and Number of Bisphosphonate Infusions

Characteristics	BRONJ (N=10)	Non-BRONJ (N=48)	Reference range	P*
Age (mean)	64.4	64.4	–	1.0
BP infusions – mean±sd	19±8	19±7	–	0.92
Average BP discontinuation (months)	9	–	–	–
Biochemical markers				
Bone formation				
Total alkaline phosphatase	101±52	85±76	40 to 150 U/L	0.54
Bone-specific alkaline phosphatase	12±6	14±8	6.5 to 22 µg/L	0.47
Osteocalcin	13±4	14±10	11 to 50 ng/mL	0.56
Bone resorption				
C-telopeptide	271±167	222±119	50 to 580 pg/mL	0.27
Angiogenesis				
Log10 Vascular-endothelial growth factor	2.92±0.38	2.41±0.35	1.78 to 2.85 pg/mL	<0.001
Endocrine				
Triiodothyronine	120±26	110±30	60 to 180 ng/dL	0.33
Thyroxine	10±2	9±2	5 to 11 µg/dL	0.40
Thyroid-stimulating hormone	2±1	2±2	0.4 to 3.0 µIU/mL	0.29
25-hydroxyvitamin D	33±14	31±10	30 to 75 µg/L	0.81
Inflammation				
C-reactive protein	34±26	13±8	0 to 10 mg/L	<0.01

* P-values were calculated from two-sample t-tests.