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Intersite reliability of vertebral bone marrow lipidomics-derived lipid composition among children with varying degrees of bone fragility undergoing routine orthopedic surgery

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Abstract

Background: Lipidomics, a branch of metabolomics, is an attractive technique to characterize bone marrow lipid composition, which may be associated with skeletal acquisition and homeostasis. However, the reliability of lipidomics-derived lipid composition of the bone marrow is unknown, especially for pediatric populations with bone fragility. The purpose of this study was to evaluate the intersite reliability and standard error of measurement (SEM) of vertebral bone marrow lipid composition at the thoracic (T11/T12) and lumbar (L1/L2) spine determined by targeted lipidomics among children with varying degrees of bone fragility undergoing routine orthopedic surgery.

Methods: Children aged between 12 and 19 years of age, with a confirmed diagnosis of adolescent idiopathic scoliosis or neuromuscular scoliosis and cerebral palsy, and undergoing routine posterior spinal fusion surgery at our institution were initially included in this study. Transpedicular vertebral body bone marrow samples were taken from thoracic (T) or lumbar (L) vertebrae. Further inclusion criteria involved having bone marrow extracted from both T11 and T12 (n=24) or L1 and L2 (n=19). Lipid composition was measured using a targeted lipidomics technique and examined as the saturated, monounsaturated, and polyunsaturated index and as individual fatty acids. Relative and absolute test-retest reliability was assessed using the intraclass correlation coefficient (ICC) and SEM.

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Declarations of interest: none.

Results: For the T11/T12 analysis: the ICC and SEM were 0.59 and 1.7% for the saturated index, 0.31 and 6.2% for the monounsaturated index, and 0.44 and 6.1% for the polyunsaturated index; the ICC showed a considerable range for individual fatty acids from 0.07 (fatty acid 20:2) to 0.82 (15:0) with 62.1% of the fatty acids having poor reliability (i.e., ICC<0.50). For the L1/L2 analysis: the ICC and SEM were 0.50 and 2.4% for the saturated index, -0.12 and 6.0% for the monounsaturated index, and 0.00 and 4.9% for the polyunsaturated index; the ICC showed a considerable range for individual fatty acids from -0.34 (18:1_n-9) to 0.88 (15:0 and 18:3_n-3) with 79.3% of the fatty acids having poor reliability.

Conclusions: The intersite test-retest reliability was poor-to-moderate for index measures and generally poor for individual fatty acids for the thoracic and lumbar spine. At this time, it is not recommended to pool bone marrow adipose tissue across vertebral sites for bone marrow adiposity research or clinical monitoring for pediatric populations with bone fragility.

Keywords

reliability; lipidomics; lipid composition; fatty acid; pediatrics

Introduction

There is growing interest in the field of bone marrow lipid biology and its potential impact on skeletal acquisition, maintenance, and homeostasis. Studies [1–9] as far back as the early 1970s [1] have established a reciprocal relationship between bone mass and marrow adiposity, which is supported by evidence that osteoblasts and bone marrow-derived adipocytes (lipid rich cells) share a common progenitor [10]. Emerging research in the field is highlighting plausible pathophysiological links between bone marrow adipose tissue and diseases of the skeletal system and other organ systems (for reviews, please see [11–16]).

While there are multiple relevant facets of bone marrow adipose physiology, bone marrow lipid composition in particular may provide mechanistic insight into the biological role that bone marrow adipose tissue may have on bone metabolism. This is because different fatty acids possess unique features that can have direct and indirect effects on bone cells that can be pro- or anti-osteogenic. For example, palmitic and stearic saturated fatty acids have been shown to be lipotoxic to bone [17, 18] and disrupt osteoblast function [19, 20], while palmitoleic, oleic, and linoleic unsaturated fatty acids have been suggested to promote the differentiation, mineralization, and survival of osteoblasts [18, 20, 21] and inhibit the differentiation and function of osteoclasts [22, 23]. Evidence has shown that the bone marrow saturated index (i.e., proportion of saturated fatty acids to total fatty acids) is associated with bone fragility [24, 25] and this index has been proposed as a novel biomarker for osteoporosis [26]. The degree of lipid saturation can be measured *in vivo* using magnetic resonance spectroscopy. However, lipidomics- a branch of metabolomics- is especially attractive for this purpose as it is able to comprehensively analyze the lipidome including the saturated, monounsaturated, and polyunsaturated index, as well as individual fatty acids, which can help to identify novel research questions and address knowledge gaps pertaining to lipid biology [27].

Growth and development is a critical period for establishing bone mass and architecture and overall peak bone mass, which has a lasting effect on bone health throughout the lifespan [28]. While it is unknown if and how bone marrow adipose tissue and lipid composition interacts with bone development in children, recent speculation is casting a light on bone marrow adiposity and lipid composition as a possible factor that may support or suppress bone acquisition depending on the environment (e.g., states of low lipid turnover, inflammation), especially for pediatric patients with varying degrees of bone fragility [16, 29]. In particular, individuals with adolescent idiopathic scoliosis (AIS) and cerebral palsy (CP) can develop progressive scoliosis and may opt to receive spinal fusion surgery to correct the scoliosis. This surgery yields extracted bone marrow tissue from the vertebral body and thus provides a highly unique opportunity to study the bone marrow lipidome in pediatric patients with varying degree of bone fragility, which can support mechanistic and therapeutic translational research with direct clinical relevance.

We have previously reported that the intrasite test-retest reliability (i.e., two separate samples taken from the same site during the same surgery) was moderate-to-good for vertebral bone marrow saturated, monounsaturated, and polyunsaturated indices, and poor-to-excellent depending on the individual fatty acid via targeted lipidomics from children with AIS and CP who underwent routine spinal fusion surgery [30]. Given the relatively small yield of bone marrow adipose tissue collected during routine surgery, researchers and clinicians may be interested in pooling the bone marrow from neighboring vertebral sites (e.g., T11-L1). However, the composition of lipids and, importantly, the reliability and absolute agreement of lipid composition from neighboring vertebrae from pediatric bone marrow is unknown. In order to assist in interpretation of bone marrow lipid composition in the research and clinical setting and to generate novel information to help power future research studies where pediatric bone marrow lipid composition is of interest, evaluation of intersite reliability is crucial. Therefore, the objective of this study was to assess the intersite test-retest reliability of targeted lipidomics-derived bone marrow lipid composition from the thoracic (T11/T12) and lumbar (L1/L2) spine among children with AIS and CP following routine orthopedic surgical care.

Methods

As there is research and clinical interest in maximizing bone marrow adipose tissue yield by pooling of tissue from multiple sites, the research question in this study addresses intersite reliability of the bone marrow lipid composition from neighboring vertebral sites along different segments of the spine after tissue extraction from routine surgery.

Participants

The Institutional Review Board approved this study. Participants who were between the ages of 12 and 19 years, had a confirmed diagnosis of AIS or CP, and were undergoing posterior spinal fusion surgery for scoliosis correction at the same institution and by the same surgeons (co-authors MSC and YL) were eligible for this research study. Parental consent and patient assent (where appropriate) was acquired in order to obtain transpedicular vertebral body bone marrow samples collected from surgery for research purposes that

would otherwise be discarded, and to acquire information regarding demographics (e.g., age at surgery, sex, race). For participants with CP, additional information was collected including the type of CP and the gross motor function classification system (GMFCS), which ranks the severity of motor impairment [31]. The participants with CP in this study had quadriplegic spastic type CP and GMFCS IV or V corresponding to severe motor impairment.

Bone marrow sample extraction

Bone marrow from T11 to L2 was extracted from the vertebral body and handled as previously described [30]. Depending on the scoliosis correction needs, some participants had their bone marrow extracted from one or multiple adjacent sites from T11 to L2 from their surgery. To evaluate intersite reliability and determine site-specific effects on reliability measures, two sets of analyses were conducted for patients who had samples from: (1) T11 and T12 or (2) L1 and L2.

Lipidomics

Obtained marrow samples were analyzed using targeted lipidomics via the Total Lipid Extraction and Thin-Layer Chromatography Cleanup assay by an experienced technician at the Michigan Nutrition Obesity Research Center Lipidomics Core, as described in detail previously [30]. All bone marrow samples were analyzed in the same batch (i.e., previously frozen until analysis in which the procedures were applied to all samples at the same time) and had not undergone a previous freeze-thaw cycle. Briefly, lipids were extracted using a modified Bligh-Dyer method of solvent partition [32] and heptadecanoic acid (17:0) was used as an internal standard. Homogenized mixtures were treated with chloroform (0.75mL) and 0.9% NaCl (0.75mL), mixed, and centrifuged for 15 minutes on a table-top centrifuge at 3,000 rpm. This led to a clear separation of layers of the mixture where the lower organic layer (chloroform) containing the lipids were transported into tubes and stored at -20°C until further analysis.

As previously described using a modified method [33], the broken down fatty acid methyl esters (FAMES) were obtained. After removing solvents under nitrogen, the crude FAMES were re-dissolved in chloroform and purified on a thin layer chromatographic (TLC) plate ($20 \times 20\text{cm}$, silica gel 60, Merck, Germany). Methyl ester bands were identified by comparing the retention flow of the authentic standard and the contents from the TLC powders were extracted with chloroform. After removing solvents under nitrogen, the methyl esters were re-dissolved in hexane. The fatty acid compositions of the lipids were analyzed using gas chromatography on an Agilent Gas Chromatography model 6890N (gas chromatography column: Agilent HP 88, 30m, 0.25mm I.D., and 0.20 μm film thickness) with flame ionization detector, an auto sampler, a temperature program of 125°C to 220°C , and Chemstation software for analysis. A calibration curve was developed using mixtures containing saturated and unsaturated carbon chain length from 12 through 24 carbons and using the peak area ratio response of each methyl ester relative to known amounts of methyl heptadecanoate. A mixture of authentic methyl esters was also run to identify the components in unknown samples by comparison of retention times. The fatty acids were quantified in regards to the amount of internal standard (17:0) added and the prepared

calibration curve. The coefficient of variation for gas chromatography analyses was between 2.3 and 3.7%.

Measures were transformed to relative abundance (%) for each individual fatty acid. We calculated the saturated, monounsaturated, and polyunsaturated indices as the proportion of the fatty acid type to the total fatty acid amount. The nomenclature used for fatty acids include the number of carbon chains and double bonds. For unsaturated fatty acids, the nomenclature also includes the number of carbon chains after the double bond, as well as *cis* (c) or *trans* (t) configuration. For example, docosapentaenoic acid, 22:5n-3, is a polyunsaturated fatty acid with 22 carbon chains and 5 double bonds with the first double bond at the 3rd carbon chain from the omega end.

Statistical analysis

Using both relative and absolute estimates, the test-retest reliability was examined by the intraclass correlation coefficient (ICC; relative) and the standard error of measurement (SEM; absolute). ICC is an ideal measure for test-retest reliability evaluation as it represents both the extent of agreement and correlation between measures [34]. The ICC and 95% confidence interval (CI) was calculated using a 2-way mixed-effects model and absolute agreement (SPSS version 24 [SPSS Inc, Chicago, IL]), which was based on guidelines for ICC selection, design, and statistical reporting for test-retest reliability [34]. As a rule of thumb, ICC>0.90 indicates excellent reliability, ICC 0.75–0.90 indicates good reliability, ICC 0.50–0.75 indicates moderate reliability, and ICC<0.50 indicates poor reliability [34]. The SEM estimates the intra-individual variability [35] and was calculated as follows: $SEM = SD_{pooled} \times (1-ICC)$, where SD_{pooled} is the pooled standard deviation.

Sensitivity analysis

Due to the small sample size, the reliability estimates are susceptible to outliers and influential observations, which were examined using both agreement and correlation methods separately for each measure. Bland-Altman plots determined the extent of agreement by plotting the difference between both samples with the average of the two sample measures [36]. The fixed bias for each measure (mean of the difference between samples) was tested statistically using a one-sample t-test and normality was examined by skewness, kurtosis, and the Shapiro-Wilk test. Potential influential observations were examined using the Cook's distance (D_i) measure, which determines the magnitude of the influence from each data point by use of residuals and leverage on the regression between measures; an increase in D_i corresponds to an increase in the extent of influence on the relationship by the data point [37]. In this study, given the small sample size, we considered a more conservative threshold of $D_i > 0.50$ compared to a more traditional 1.0 to suggest a potential of an influential observation [38]. While the ICC represents both agreement and correlation between measures [34], Bland-Altman plots and D_i for model diagnostics assesses either and not both concurrently. For lipid composition measures that suggested potential outliers or influential observations (e.g., $D_i > 0.50$), the data point(s) were removed and the ICC was re-examined to determine if these observations affected the reliability estimates, which was assessed qualitatively.

Data were analyzed using SPSS statistical package version 24 (SPSS Inc, Chicago, IL). An α level of 0.05 was used as the threshold to determine statistical significance.

Results

Descriptive characteristics of the 24 participants (all AIS) for the T11/T12 analysis and 19 participants (n=3 CP) for the L1/L2 analysis are presented in Table 1. The three participants with CP had spastic quadriplegic type and were classified as GMFCS IV (n=2) or V (n=1).

T11/T12 test-retest reliability

The average ($\pm$ standard deviation), ICC (95% CI) and SEM of lipid composition measures from T11/T12 bone marrow are shown in Table 2, which is presented based on index vs. individual fatty acids, type of fatty acid (e.g., monounsaturated), and the ICC point estimate. Based on the average of the T11 sample (average of T12 sample), 31.8% (30.8%) of the bone marrow lipids consisted of saturated fatty acids, and 40.0% (39.6%) and 28.1% (29.7%) consisted of monounsaturated and polyunsaturated fatty acids, respectively.

For the index measures, the ICC (95% CI) was 0.59 (0.25, 0.80) for the saturated index, suggesting moderate (poor to good) reliability, and 0.31 (−0.11, 0.64) and 0.44 (0.05, 0.71) for the monounsaturated and polyunsaturated indices, respectively, suggesting poor (to moderate) reliability. The SEM was 1.7%, 6.2%, and 6.1% for the saturated, monounsaturated, and polyunsaturated index, respectively. For the index measures, Figure 1 demonstrates the relationship (A-C, scatter plots) and agreement (D-F, Bland-Altman plots) between bone marrow samples. Based on the Bland-Altman plots, the average bias was minimal (near zero) for each index measure. While there was no evidence of fixed bias for the monounsaturated or polyunsaturated index measures (both $P>0.40$ using a one-sample t-test), there was evidence of fixed bias for the saturated index measure ($P=0.034$).

The relative abundance of 13:0 was too low to detect and assess reliability. For all other fatty acids, the ICC (95% CI) exhibited a considerable range from 0.07 (−0.35, 0.47) to 0.82 (0.62, 0.92). Based on the ICC point estimate and excluding 13:0, 0 had excellent reliability, 4 had good reliability (13.8%), 7 had moderate reliability (24.1%), and 18 had poor reliability (62.1%).

L1/L2 test-retest reliability

The average ($\pm$ standard deviation), ICC (95% CI) and SEM of lipid composition measures from L1/L2 bone marrow are presented in Table 3. Based on the average of the L1 sample (average of L2 sample), 31.8% (31.2%) of the bone marrow lipids consisted of saturated fatty acids, and 39.3% (39.9%) and 29.0% (28.9%) consisted of monounsaturated and polyunsaturated fatty acids, respectively.

For the index measures, the ICC (95% CI) was 0.50 (0.06, 0.77) for the saturated index, suggesting moderate (poor to good) reliability, and −0.12 (−0.56, 0.36) and 0.00 (−0.46, 0.45) for the monounsaturated and polyunsaturated indices, respectively, suggesting poor reliability. The SEM was 2.4%, 6.0%, and 4.9% for the saturated, monounsaturated, and polyunsaturated index, respectively. For the index measures, Figure 2 demonstrates the

relationship (A-C, scatter plots) and agreement (D-F, Bland-Altman plots) between bone marrow samples. Based on the Bland-Altman plots, the average bias was minimal (near zero) for each index measure and there was no evidence of fixed bias (all $P>0.55$ using a one-sample t-test).

The relative abundance of 13:0 was too low to detect and assess reliability. For all other fatty acids, the ICC (95% CI) exhibited a considerable range from -0.34 ($-0.73, 0.16$) to 0.86 ($0.68, 0.94$). Based on the ICC point estimate and excluding 13:0, 0 had excellent reliability, 3 had good reliability (10.3%), 3 had moderate reliability (10.3%), and 23 had poor reliability (79.3%).

Sensitivity analysis

There was evidence of one to two potential influential observations for 13 individual fatty acids for the T11/T12 analysis and the polyunsaturated index and 20 individual fatty acids for the L1/L2 analysis. The ICC (95% CI) and SEM after removing the potential influential observation(s) is presented in Table 4 for both the T11/T12 and L1/L2 analysis.

For the T11/T12 analysis, the ICC point estimate increased in 6 of the fatty acids ($n=5$ saturated, $n=1$ monounsaturated), decreased in 5 of the fatty acids ($n=2$ monounsaturated, $n=3$ polyunsaturated), and 2 fatty acids exhibited negligible change ($n=1$ saturated, $n=1$ polyunsaturated). With these changes, 23 out of the 29 fatty acids (79.3%) had poor reliability (i.e., $ICC<0.50$), which is worse than the primary analysis (62.1%).

For the L1/L2 analysis, the ICC point estimate decreased in the polyunsaturated index, increased in 5 of the fatty acids ($n=3$ saturated, $n=2$ monounsaturated), decreased in 10 of the fatty acids ($n=4$ saturated, $n=4$ monounsaturated, $n=2$ polyunsaturated), and 5 fatty acids exhibited negligible change ($n=1$ saturated, $n=1$ monounsaturated, $n=3$ polyunsaturated). With these changes, the polyunsaturated index and 25 out of the 29 fatty acids (86.2%) had poor reliability (i.e., $ICC<0.50$), which is slightly worse than the primary analysis (79.3%).

Discussion

The goal of this study was to assess intersite test-retest reliability of thoracic and lumbar bone marrow saturated and unsaturated indices in targeted lipidomics from children with AIS and CP using bone marrow obtained during routine orthopedic surgery. The findings suggest intersite test-retest reliability is poor-to-moderate for these indices. Additionally, while there was a large range of ICC values, the majority of individual fatty acids exhibited poor intersite test-retest reliability for the thoracic (62.1%) and lumbar (79.3%) sites, which became worse after removing potential influential observations (thoracic, 79.3%; lumbar, 86.2%). The results from this study have important logistical indications for research and clinical interests. It is not yet fully understood if and how bone marrow adiposity and its lipid composition contributes to skeletal acquisition or metabolism among pediatric populations. Based on the study findings, pooling bone marrow from neighboring vertebral sites may increase the heterogeneity of an already heterogeneous tissue, thus complicating and diluting experimentation and its interpretation in the research and clinical setting.

The suboptimal intersite reliability estimates from this study are in contrast to our previous study that examined intrasite reliability in the same region of the spine in a sample of 10–19 year olds with AIS and CP (n=16) [30]. For the intrasite reliability study, two separate, but immediately adjacent, marrow samples were obtained and analyzed from the same vertebrae. Both studies, [30] and the current study, used the same methods, the lipidomics analysis was performed on the same batch, and all samples had not undergone a previous freeze-thaw cycle. Specifically, we have previously reported that the intrasite reliability was moderate-to-excellent for the saturated and unsaturated indices and poor-to-excellent for individual fatty acids, where 34.5% of the individual fatty acids had good-to-excellent reliability, 37.9% had moderate reliability, and 27.6% had poor reliability [30]. In the current study, 62.1% to 86.2% of the individual fatty acids exhibited poor intersite reliability, depending on the site (T11/T12 vs. L1/L2) and exclusion of influential observations.

The subpar reliability estimates observed in the current study are likely due to many reasons. One reason may be the low relative abundance of some fatty acids, as previously discussed in depth [30]. In addition, there are many factors associated with AIS and CP that may influence bone marrow lipid composition, such as diet, endocrine alterations, puberty, diseases, surgical history, and medications, among other factors [16, 39, 40]. These factors may help to explain, in part, the difference in reliability estimates between our previous intrasite analysis [30] and the current intersite analysis. For example, factors that influence bone marrow lipid composition may be less likely to influence tissue heterogeneity when the tissue is collected from the same vertebral site within the individual, thus leading to relatively higher reliability estimates. On the other hand, different vertebral sites may be more sensitive to tissue heterogeneity within the individual under routine surgical conditions, thus leading to lower reliability estimates.

Magnetic resonance based studies have shown that the bone marrow fat fraction increases along the caudal axis of the spine among healthy young and older adults [41, 42]. Specifically, Ruschke and colleagues [43] found a consistent elevation of bone marrow fat fraction across vertebrae in the caudal direction, with the rate of change (i.e., slope) at ~1% per vertebrae for 3–18 year olds. However, greater fat infiltration may not correspond to a consistent alteration in the relative abundance of individual fatty acids, and therefore, lipid composition. While our study was not designed to examine differences in the lipid composition along the spine, there was little evidence to suggest a difference in the index measures from the relatively narrow region of T11 to L2. This may suggest that the pattern of lipid composition remains relatively stable from T11 to L2. Although, given the focus of this study, the lack of overall marrow fat fraction, and the lack of research regarding vertebral lipid composition among pediatric populations, speculation regarding patterns of lipid composition measures along the spine based on this study is limited. Future research designed to address this knowledge gap is needed. Nevertheless, the unique anatomical and functional (e.g., weight bearing) aspects and the extent of bone marrow adipose tissue infiltration of vertebrae along the spine may create a microenvironment that establishes its own lipid composition based on the metabolic or biological needs or consequences (e.g., from bone and/or endocrine complications associated with AIS or CP), thus potentially explaining, in part, the generally poor intersite reliability estimates in this study.

Based on our intrasite analysis [30], we have previously provided evidence-based information for handling and storing bone marrow tissue for lipid composition analysis to enhance rigor and interpretation. Given the findings from this study, if vertebral bone marrow lipid composition is a primary measure of interest for pediatric populations with bone fragility, we recommend not pooling vertebral bone marrow from neighboring vertebrae, at least until more is known about why there is such marked intersite heterogeneity. However, the pediatric bone marrow lipid composition profile may or may not be associated with the function of the bone marrow microenvironment, such as inflammatory or adipokine secretion, which would be the predominant focus of research studies interested in delineating the effect of bone marrow on bone metabolism [16]. Future studies are needed to determine the biological effect and secretory profiles of bone marrow and their reliability estimates from the same and different sites.

A major strength of the present work is that this is the first study to assess the intersite test-retest reliability of lipidomics-derived bone marrow lipid composition among pediatric populations with varying degrees of bone fragility. Obtaining bone marrow from pediatrics for research is rare and using human tissue supports data acquisition that has direct clinical relevance. As the field of bone marrow adiposity and its effect on bone metabolism grows, this study provides novel insights about the utility and interpretability of bone marrow lipidomics among pediatric populations with bone fragility to guide future research approaches. One limitation of this study is the small sample size. However, our previous intrasite study [30], which exhibited higher reliability estimates and had an even smaller sample size (n=16), utilized the same methodology and the lipid composition was analyzed in the same batch as the samples from the current study. Obtaining a large sample size is not feasible because of the invasive process to obtain bone marrow. As studies centered on clinical pediatric populations (e.g., AIS, CP) often have relatively small sample sizes due to logistical challenges (e.g., lower recruitment pool of potential participants), study findings from our sample have practical and real world implications in terms of research investigation of tissue and biological outcomes.

Conclusion

This study found that intersite test-retest reliability was poor-to-moderate for thoracic and lumbar saturated and unsaturated indices and generally poor for individual fatty acids using targeted lipidomics from pediatric populations with varying degrees of bone fragility where bone marrow was obtained during routine orthopedic surgical conditions. While intrasite reliability is relatively higher for vertebral bone marrow lipid composition measures [30], we urge caution when considering pooling bone marrow from different vertebrae when a measure of interest includes a lipid composition measure.

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Abbreviations:

AIS	adolescent idiopathic scoliosis
CP	cerebral palsy
ICC	intraclass correlation coefficient
SEM	standard error of measurement
FAME	fatty acid methyl esters
TLC	thin layer chromatography

References

- [1]. Meunier P, Aaron J, Edouard C, Vignon G, Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies, *Clinical orthopaedics and related research* 80 (1971) 147–54. [PubMed: 5133320]
- [2]. Minaire P, Edouard C, Arlot M, Meunier PJ, Marrow changes in paraplegic patients, *Calcified Tissue Int* 36(3) (1984) 338–40.
- [3]. Martin RB, Zissimos SL, Relationships between marrow fat and bone turnover in ovariectomized and intact rats, *Bone* 12(2) (1991) 123–31. [PubMed: 2064840]
- [4]. Di Iorgi N, Mo AO, Grimm K, Wren TA, Dorey F, Gilsanz V, Bone acquisition in healthy young females is reciprocally related to marrow adiposity, *The Journal of clinical endocrinology and metabolism* 95(6) (2010) 2977–82. [PubMed: 20392872]
- [5]. Di Iorgi N, Rosol M, Mittelman SD, Gilsanz V, Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults, *J Clin Endocrinol Metab* 93(6) (2008) 2281–6. [PubMed: 18381577]
- [6]. Wren TA, Chung SA, Dorey FJ, Bluml S, Adams GB, Gilsanz V, Bone marrow fat is inversely related to cortical bone in young and old subjects, *The Journal of clinical endocrinology and metabolism* 96(3) (2011) 782–6. [PubMed: 21177790]
- [7]. Shen W, Velasquez G, Chen J, Jin Y, Heymsfield SB, Gallagher D, Pi-Sunyer FX, Comparison of the relationship between bone marrow adipose tissue and volumetric bone mineral density in children and adults, *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* 17(1) (2014) 163–9. [PubMed: 23522982]
- [8]. Cohen A, Dempster DW, Stein EM, Nickolas TL, Zhou H, McMahon DJ, Muller R, Kohler T, Zwahlen A, Lappe JM, Young P, Recker RR, Shane E, Increased marrow adiposity in premenopausal women with idiopathic osteoporosis, *The Journal of clinical endocrinology and metabolism* 97(8) (2012) 2782–91. [PubMed: 22701013]
- [9]. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M, Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis, *Biogerontology* 2(3) (2001) 165–71. [PubMed: 11708718]
- [10]. David V, Martin A, Lafage-Proust MH, Malaval L, Peyroche S, Jones DB, Vico L, Guignandon A, Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis, *Endocrinology* 148(5) (2007) 2553–62. [PubMed: 17317771]
- [11]. Lecka-Czernik B, Marrow fat metabolism is linked to the systemic energy metabolism, *Bone* 50(2) (2012) 534–9. [PubMed: 21757043]

- [12]. Fazeli PK, Horowitz MC, MacDougald OA, Scheller EL, Rodeheffer MS, Rosen CJ, Klibanski A, Marrow fat and bone--new perspectives, *J Clin Endocrinol Metab* 98(3) (2013) 935–45. [PubMed: 23393168]
- [13]. Devlin MJ, Rosen CJ, The bone-fat interface: basic and clinical implications of marrow adiposity, *Lancet Diabetes Endocrinol* 3(2) (2015) 141–7. [PubMed: 24731667]
- [14]. During A, Penel G, Hardouin P, Understanding the local actions of lipids in bone physiology, *Prog Lipid Res* 59 (2015) 126–46. [PubMed: 26118851]
- [15]. Scheller EL, Cawthorn WP, Burr AA, Horowitz MC, MacDougald OA, Marrow Adipose Tissue: Trimming the Fat, *Trends Endocrinol Metab* 27(6) (2016) 392–403. [PubMed: 27094502]
- [16]. Whitney DG, Peterson MD, Devlin MJ, Caird MS, Hurvitz EA, Modlesky CM, Bone Marrow Fat Physiology in Relation to Skeletal Metabolism and Cardiometabolic Disease Risk in Children With Cerebral Palsy, *Am J Phys Med Rehabil* 97(12) (2018) 911–919. [PubMed: 29894311]
- [17]. Alsahli A, Kieffhaber K, Gold T, Muluke M, Jiang H, Cremers S, Schulze-Spate U, Palmitic Acid Reduces Circulating Bone Formation Markers in Obese Animals and Impairs Osteoblast Activity via C16-Ceramide Accumulation, *Calcif Tissue Int* 98(5) (2016) 511–9. [PubMed: 26758875]
- [18]. Gillet C, Spruyt D, Rigutto S, Dalla Valle A, Berlier J, Louis C, Debier C, Gaspard N, Malaisse WJ, Gangji V, Rasschaert J, Oleate Abrogates Palmitate-Induced Lipotoxicity and Proinflammatory Response in Human Bone Marrow-Derived Mesenchymal Stem Cells and Osteoblastic Cells, *Endocrinology* 156(11) (2015) 4081–93. [PubMed: 26327577]
- [19]. Gunaratnam K, Vidal C, Gimble JM, Duque G, Mechanisms of palmitate-induced lipotoxicity in human osteoblasts, *Endocrinology* 155(1) (2014) 108–16. [PubMed: 24169557]
- [20]. Elbaz A, Wu X, Rivas D, Gimble JM, Duque G, Inhibition of fatty acid biosynthesis prevents adipocyte lipotoxicity on human osteoblasts in vitro, *J Cell Mol Med* 14(4) (2010) 982–91. [PubMed: 19382912]
- [21]. Platt ID, El-Sohemy A, Regulation of osteoblast and adipocyte differentiation from human mesenchymal stem cells by conjugated linoleic acid, *J Nutr Biochem* 20(12) (2009) 956–64. [PubMed: 19019668]
- [22]. Rahman MM, Halade GV, Williams PJ, Fernandes G, t10c12-CLA maintains higher bone mineral density during aging by modulating osteoclastogenesis and bone marrow adiposity, *J Cell Physiol* 226(9) (2011) 2406–14. [PubMed: 21660964]
- [23]. van Heerden B, Kasonga A, Kruger MC, Coetzee M, Palmitoleic Acid Inhibits RANKL-Induced Osteoclastogenesis and Bone Resorption by Suppressing NF-kappaB and MAPK Signalling Pathways, *Nutrients* 9(5) (2017).
- [24]. Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC, Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study, *J Magn Reson Imaging* 22(2) (2005) 279–85. [PubMed: 16028245]
- [25]. Patsch JM, Li X, Baum T, Yap SP, Karampinos DC, Schwartz AV, Link TM, Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures, *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 28(8) (2013) 1721–8.
- [26]. Di Pietro G, Capuani S, Manenti G, Vinicola V, Fusco A, Baldi J, Scimeca M, Hagberg G, Bozzali M, Simonetti G, Tarantino U, Bone Marrow Lipid Profiles from Peripheral Skeleton as Potential Biomarkers for Osteoporosis: A 1H-MR Spectroscopy Study, *Acad Radiol* 23(3) (2016) 273–83. [PubMed: 26774740]
- [27]. Lam SM, Shui G, Lipidomics as a principal tool for advancing biomedical research, *J Genet Genomics* 40(8) (2013) 375–90. [PubMed: 23969247]
- [28]. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA, Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass, *J Bone Miner Res* 26(8) (2011) 1729–39. [PubMed: 21520276]
- [29]. Shiran SI, Shabtai L, Ben-Sira L, Ovadia D, Wientroub S, T1-weighted MR imaging of bone marrow pattern in children with adolescent idiopathic scoliosis: a preliminary study, *J Child Orthop* 12(2) (2018) 181–186. [PubMed: 29707058]

- [30]. Whitney DG, Devlin MJ, Alford AI, Modlesky CM, Peterson MD, Li Y, Caird MS, Test-retest reliability and correlates of vertebral bone marrow lipid composition by lipidomics among children with varying degrees of bone fragility, *JBMR Plus* In Press (2020).
- [31]. Wood E, Rosenbaum P, The gross motor function classification system for cerebral palsy: a study of reliability and stability over time, *Developmental medicine and child neurology* 42(5) (2000) 292–6. [PubMed: 10855648]
- [32]. Bligh EG, Dyer WJ, A rapid method of total lipid extraction and purification, *Can J Biochem Physiol* 37(8) (1959) 911–7. [PubMed: 13671378]
- [33]. Morrison WR, Smith LM, Preparation of Fatty Acid Methyl Esters + Dimethylacetals from Lipids with Boron Fluoride-Methanol, *J Lipid Res* 5(4) (1964) 600–&. [PubMed: 14221106]
- [34]. Koo TK, Li MY, A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research, *J Chiropr Med* 15(2) (2016) 155–63. [PubMed: 27330520]
- [35]. Bueno-Gracia E, Malo-Urries M, Ruiz-de-Escudero-Zapico A, Rodriguez-Marco S, Jimenez-Del-Barrio S, Shacklock M, Estebanez-de-Miguel E, Tricas-Moreno JM, Reliability of measurement of the carpal tunnel and median nerve in asymptomatic subjects with ultrasound, *Musculoskelet Sci Pract* 32 (2017) 17–22. [PubMed: 28800435]
- [36]. Bland JM, Altman DG, Statistical methods for assessing agreement between two methods of clinical measurement, *Lancet* 1(8476) (1986) 307–10. [PubMed: 2868172]
- [37]. Cook RD, Influential Observations in Linear Regression, *Journal of the American Statistical Association* 74(365) (1979) 169–174.
- [38]. Cook DR, *Residuals and Influence in Regression*, Chapman & Hall, New York, NY, 1982.
- [39]. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ, Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment, *Nature* 460(7252) (2009) 259–63. [PubMed: 19516257]
- [40]. Menagh PJ, Turner RT, Jump DB, Wong CP, Lowry MB, Yakar S, Rosen CJ, Iwaniec UT, Growth hormone regulates the balance between bone formation and bone marrow adiposity, *J Bone Miner Res* 25(4) (2010) 757–68. [PubMed: 19821771]
- [41]. Baum T, Yap SP, Dieckmeyer M, Ruschke S, Eggers H, Kooijman H, Rummeny EJ, Bauer JS, Karampinos DC, Assessment of whole spine vertebral bone marrow fat using chemical shift-encoding based water-fat MRI, *J Magn Reson Imaging* 42(4) (2015) 1018–23. [PubMed: 25639780]
- [42]. Hassan EB, Demontiero O, Vogrin S, Ng A, Duque G, Marrow Adipose Tissue in Older Men: Association with Visceral and Subcutaneous Fat, Bone Volume, Metabolism, and Inflammation, *Calcified Tissue Int* 103(2) (2018) 164–174.
- [43]. Ruschke S, Pokorney A, Baum T, Eggers H, Miller JH, Hu HH, Karampinos DC, Measurement of vertebral bone marrow proton density fat fraction in children using quantitative water-fat MRI, *MAGMA* 30(5) (2017) 449–460. [PubMed: 28382554]

Figure 1. Scatter plots show the correlation (A-C) and Bland-Altman plots show the agreement (D-F) for the lipid composition index measures for T11 and T12 marrow samples. In A-C, the dotted line represents the line of identity and the solid line represents the regression line. In D-F, the dotted lines represent the mean difference between marrow sample pairs ± 1.96 standard deviation.

Figure 2.

Scatter plots show the correlation (A-C) and Bland-Altman plots show the agreement (D-F) for the lipid composition index measures for L1 and L2 marrow samples. In A-C, the dotted line represents the line of identity and the solid line represents the regression line. In D-F, the dotted lines represent the mean difference between marrow sample pairs ± 1.96 standard deviation.

Table 1.

Descriptive characteristics of participants by vertebral region.

	Thoracic	Lumbar
Adolescent idiopathic scoliosis, n (%)	24 (100)	16 (84.2)
Cerebral palsy, n (%)	0 (0)	3 (15.8)
GMFCS, n		
I-III	0	0
IV	0	2
V	0	1
Age, mean (SD)	14.9 (1.7)	15.4 (1.5)
Sex, n (%)		
Female	20 (83.3)	12 (63.2)
Male	4 (16.7)	7 (36.8)
Race, n (%)		
White	19 (79.2)	15 (78.9)
Black	4 (16.7)	2 (10.5)
Biracial*	0 (0)	1 (5.3)
Declined/missing	1 (4.1)	1 (5.3)
Height (cm), mean (SD)	161.8 (10.3)	162.8 (13.9)
Body mass (kg), mean (SD)	49.3 (10.6)	50.1 (12.2)
Body mass index (kg/m ²), mean (SD)	18.7 (3.2)	18.6 (2.9)
Age- and sex-based BMI %, mean (SD)	31.3 (32.0)	28.8 (28.2)

GMFCS, gross motor function classification system; SD, standard deviation.

* Identified race as Black and White.

Table 2.

Relative and absolute test-retest reliability estimates of lipid composition measures from 24 thoracic vertebral bone marrow sample pairs.

	T11 Mean ± SD	T12 Mean ± SD	ICC (95% CI)	SEM
Index measures	(%)	(%)	ICC (95% CI)	(%)
Saturated index	31.8 ± 2.6	30.8 ± 2.7	0.59 (0.25, 0.80)	1.7
Monounsaturated index	40.0 ± 7.0	39.6 ± 7.9	0.31 (−0.11, 0.64)	6.2
Polyunsaturated index	28.1 ± 7.3	29.7 ± 9.1	0.44 (0.05, 0.71)	6.1
Saturated fatty acids				
15:0	0.12 ± 0.11	0.11 ± 0.11	0.82 (0.62, 0.92)	0.05
16:0	20.78 ± 2.06	20.07 ± 2.33	0.59 (0.26, 0.80)	1.41
14:0	1.17 ± 0.74	1.24 ± 0.82	0.49 (0.11, 0.74)	0.56
24:0	0.36 ± 0.24	0.29 ± 0.21	0.31 (−0.09, 0.63)	0.19
18:0	8.73 ± 1.70	8.39 ± 1.55	0.30 (−0.11, 0.62)	1.36
20:0	0.18 ± 0.07	0.19 ± 0.07	0.25 (−0.18, 0.59)	0.06
12:0	0.02 ± 0.04	0.03 ± 0.05	0.21 (−0.21, 0.56)	0.04
19:0	0.18 ± 0.05	0.16 ± 0.04	0.18 (−0.22, 0.53)	0.04
21:0	0.05 ± 0.03	0.04 ± 0.02	0.18 (−0.24, 0.54)	0.02
22:0	0.23 ± 0.12	0.24 ± 0.19	0.09 (−0.34, 0.48)	0.15
13:0	<0.01 ± <0.01	0.01 ± 0.01	NA	NA
Monounsaturated fatty acids				
18:1_n-7	6.77 ± 8.58	9.45 ± 10.86	0.78 (0.54, 0.90)	4.56
18:1_n-9	29.88 ± 10.07	26.77 ± 13.27	0.57 (0.24, 0.79)	7.65
14:1_n-5	0.12 ± 0.12	0.10 ± 0.08	0.56 (0.22, 0.78)	0.07
16:1_n-7t	0.30 ± 0.06	0.29 ± 0.08	0.51 (0.13, 0.75)	0.05
16:1_n-7c	1.96 ± 0.69	1.97 ± 0.77	0.42 (0.01, 0.70)	0.56
20:1	0.59 ± 0.15	0.58 ± 0.17	0.27 (−0.15, 0.61)	0.14
22:1	0.06 ± 0.03	0.06 ± 0.03	0.15 (−0.28, 0.52)	0.03
24:1	0.36 ± 0.26	0.37 ± 0.28	0.08 (−0.35, 0.47)	0.26
Polyunsaturated fatty acids				
18:3_n-3	0.75 ± 0.31	0.83 ± 0.34	0.81 (0.60, 0.92)	0.14
18:3_n-6	0.11 ± 0.11	0.12 ± 0.12	0.80 (0.59, 0.91)	0.05
20:5_n-3	0.06 ± 0.09	0.10 ± 0.15	0.74 (0.47, 0.88)	0.06
22:6_n-3	0.38 ± 0.28	0.49 ± 0.49	0.54 (0.20, 0.77)	0.26
18:2_n-6	22.30 ± 4.59	23.48 ± 5.61	0.52 (0.16, 0.76)	3.53
20:3_n-6	0.52 ± 0.37	0.55 ± 0.45	0.39 (−0.01, 0.69)	0.32
20:4_n-6	2.92 ± 1.97	3.01 ± 2.40	0.27 (−0.16, 0.61)	1.87
22:5_n-3	0.40 ± 0.20	0.41 ± 0.25	0.26 (−0.16, 0.60)	0.19
22:2_n-6	0.03 ± 0.02	0.03 ± 0.03	0.16 (−0.27, 0.53)	0.02
22:4_n-6	0.49 ± 0.27	0.44 ± 0.23	0.14 (−0.28, 0.51)	0.23
20:2	0.19 ± 0.12	0.19 ± 0.12	0.07 (−0.35, 0.47)	0.12

SD, standard deviation; ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of measurement; NA, not available (due to low detection).

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Table 3.

Relative and absolute test-retest reliability estimates of lipid composition measures from 19 lumbar vertebral bone marrow sample pairs.

	L1 Mean ± SD	L2 Mean ± SD	ICC (95% CI)	SEM
Index measures	(%)	(%)	ICC (95% CI)	(%)
Saturated index	31.8 ± 2.9	31.2 ± 4.0	0.50 (0.06, 0.77)	2.4
Monounsaturated index	39.3 ± 4.9	39.9 ± 6.5	−0.12 (−0.56, 0.36)	6.0
Polyunsaturated index	29.0 ± 4.6	28.9 ± 5.2	0.00 (−0.46, 0.45)	4.9
Saturated fatty acids				
15:0	0.08 ± 0.10	0.08 ± 0.10	0.86 (0.68, 0.94)	0.04
20:0	0.19 ± 0.08	0.18 ± 0.07	0.80 (0.55, 0.92)	0.03
16:0	20.43 ± 1.48	20.24 ± 2.43	0.73 (0.42, 0.89)	1.02
14:0	1.06 ± 0.67	1.05 ± 0.62	0.41 (−0.06, 0.72)	0.50
12:0	0.03 ± 0.05	0.05 ± 0.09	0.29 (−0.15, 0.65)	0.06
18:0	9.08 ± 1.93	8.68 ± 1.80	0.21 (−0.27, 0.60)	1.66
24:0	0.41 ± 0.23	0.41 ± 0.24	0.18 (−0.31, 0.59)	0.21
22:0	0.24 ± 0.10	0.25 ± 0.14	0.15 (−0.34, 0.57)	0.11
21:0	0.07 ± 0.08	0.04 ± 0.02	0.13 (−0.31, 0.53)	0.05
19:0	0.20 ± 0.13	0.18 ± 0.07	0.06 (−0.42, 0.50)	0.10
13:0	<0.01 ± 0.01	<0.01 ± <0.01	NA	NA
Monounsaturated fatty acids				
16:1_n-7t	0.30 ± 0.06	0.31 ± 0.08	0.47 (0.02, 0.76)	0.05
16:1_n-7c	1.91 ± 0.69	2.05 ± 0.69	0.46 (0.02, 0.75)	0.51
14:1_n-5	0.09 ± 0.07	0.10 ± 0.07	0.40 (−0.07, 0.72)	0.05
20:1	0.59 ± 0.12	0.62 ± 0.22	0.32 (−0.14, 0.67)	0.14
22:1	0.07 ± 0.03	0.06 ± 0.03	0.29 (−0.20, 0.66)	0.03
24:1	0.48 ± 0.35	0.49 ± 0.28	0.23 (−0.27, 0.61)	0.28
18:1_n-7	4.60 ± 0.87	5.98 ± 7.30	−0.04 (−0.48, 0.42)	4.17
18:1_n-9	31.20 ± 5.24	30.31 ± 8.78	−0.34 (−0.73, 0.16)	8.11
Polyunsaturated fatty acids				
18:3_n-3	1.03 ± 0.84	1.38 ± 2.06	0.86 (0.68, 0.94)	0.54
22:4_n-6	0.55 ± 0.27	0.54 ± 0.30	0.69 (0.35, 0.87)	0.16
20:3_n-6	0.59 ± 0.32	0.51 ± 0.37	0.58 (0.19, 0.81)	0.22
18:3_n-6	0.10 ± 0.06	0.08 ± 0.05	0.44 (0.02, 0.73)	0.04
22:2_n-6	0.04 ± 0.05	0.03 ± 0.02	0.29 (−0.17, 0.65)	0.03
22:6_n-3	0.42 ± 0.32	0.40 ± 0.28	0.25 (−0.24, 0.63)	0.26
20:4_n-6	2.89 ± 1.42	2.90 ± 1.44	0.23 (−0.26, 0.62)	1.25
18:2_n-6	22.53 ± 2.96	22.24 ± 2.62	0.13 (−0.36, 0.55)	2.60
22:5_n-3	0.47 ± 0.23	0.50 ± 0.35	0.03 (−0.44, 0.48)	0.29
20:5_n-3	0.10 ± 0.15	0.12 ± 0.27	−0.09 (−0.51, 0.37)	0.22
20:2	0.24 ± 0.12	0.20 ± 0.13	−0.08 (−0.51, 0.38)	0.13

SD, standard deviation; ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of measurement; NA, not available (due to low detection).

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Table 4.

Relative and absolute test-retest reliability estimates of lipid composition measures from vertebral bone marrow sample pairs after removing potential influential observations.

	Thoracic		Lumbar	
	ICC (95% CI)	SEM (%)	ICC (95% CI)	SEM (%)
Index measures				
Saturated index				
Monounsaturated index				
Polyunsaturated index			−0.23 (−0.66, 0.27)	4.4
Saturated fatty acids				
15:0	0.90 (0.78, 0.96)	0.04		
16:0	0.57 (0.22, 0.79)	1.15	0.66 (0.29, 0.86)	0.76
14:0			0.03 (−0.46, 0.49)	0.49
24:0	0.41 (0.01, 0.70)	0.15	−0.05 (−0.53, 0.43)	0.20
18:0			0.21 (−0.27, 0.60)	1.60
20:0	0.40 (−0.02, 0.69)	0.05		
12:0			0.39 (−0.12, 0.73)	0.03
19:0	0.54 (0.17, 0.77)	0.02	0.23 (−0.24, 0.61)	0.04
21:0	0.43 (0.02, 0.71)	0.01	0.44 (0.00, 0.74)	0.01
22:0			−0.08 (−0.55, 0.41)	0.12
13:0				
Monounsaturated fatty acids				
18:1_n-7	0.51 (0.15, 0.76)	4.30	0.20 (−0.26, 0.60)	0.75
18:1_n-9			−0.19 (−0.62, 0.31)	6.28
14:1_n-5			−0.08 (−0.52, 0.39)	0.05
16:1_n-7t	0.34 (−0.09, 0.66)	0.05		
16:1_n-7c			−0.18 (−0.60, 0.30)	0.50
20:1	0.42 (0.02, 0.70)	0.11	0.32 (−0.14, 0.67)	0.09
22:1			−0.40 (−0.78, 0.11)	0.03
24:1			0.07 (−0.55, 0.42)	0.18
Polyunsaturated fatty acids				
18:3_n-3				
18:3_n-6	0.41 (0.00, 0.70)	0.05		
20:5_n-3	0.36 (−0.02, 0.66)	0.05		
22:6_n-3	0.41 (0.01, 0.69)	0.24	0.25 (−0.24, 0.63)	0.21
18:2_n-6				
20:3_n-6			0.40 (−0.04, 0.72)	0.23
20:4_n-6				
22:5_n-3			0.03 (−0.44, 0.48)	0.16
22:2_n-6	0.19 (−0.22, 0.55)	0.02	0.29 (−0.17, 0.65)	0.01
22:4_n-6			0.27 (−0.24, 0.65)	0.16
20:2				

SD, standard deviation; ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of measurement.

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