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Impact of frequent apheresis blood donation on bone density: A prospective, longitudinal, randomized, controlled trial☆

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ABSTRACT

Background: Blood for transfusion is lifesaving and essential to many elements of modern medical practice. The global blood supply relies on volunteer blood donors. Apheresis is increasingly used to collect blood and requires anticoagulant to prevent extracorporeal coagulation. Citrate, the standard apheresis anticoagulant, chelates ionized calcium with consequent perturbations of serum calcium, parathyroid hormone, vitamin D, and markers of bone remodeling in donors. Cross-sectional studies of bone mineral density (BMD) among apheresis donors exhibit conflicting results.

Methods: The longitudinal, randomized, controlled ALTRUYST trial (NCT02655055) was undertaken to determine whether BMD declined following high frequency apheresis blood donation over 1 year. The study was powered at 80% to detect the primary outcome of a 3% decline in BMD. Subjects new to apheresis agreed to make ≥20 apheresis donations in a one-year period and were randomized to treatment (high frequency apheresis) or control (no apheresis). Dual-energy x-ray absorptiometry was performed before and after participation. Two-sided *t*-test and multivariable logistic regression were used to assess outcomes.

Findings: Mean lumbar spine BMD did not change during the study among control donors (-0.002 g/cm^2, 95%CI [-0.020, 0.016], $p = 0.78$), or among donors in the apheresis arm (mean change = 0.007 g/cm^2, 95%CI [-0.005, 0.018], $p = 0.24$). Mean total hip BMD did not change for control donors (mean change = 0.002 g/cm^2, 95%CI [-0.006, 0.009], $p = 0.63$) or apheresis donors (-0.004 g/cm^2, 95%CI [-0.10, 0.002], $p = 0.16$). Tests for differences in proportions of donors with change in BMD exceeding the least significant change at the lumbar spine in either a positive [8 apheresis (31%), 4 control (27%), $p = 0.78$] or negative direction [4 apheresis (15%), 5 control (33%)] were statistically non-significant ($p = 0.18$). Proportional increases [0 apheresis (0%), 1 control (7%), $p = 0.18$] and decreases [3 apheresis (12%), 1 control (14%)] were also not significantly different at the total hip ($p = 0.61$).

Interpretation: ALTRUYST is the first longitudinal trial to demonstrate that apheresis blood collection guidelines in the United States adequately protect the skeletal health of male volunteer blood donors.

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Research in context

Evidence before this study

Central to the safety and availability of the global blood supply is the community of volunteer blood donors. Cross-sectional studies have reported that intermittent exposure to citrate through apheresis blood donation is associated with significant declines in donor bone mineral density (BMD). In contrast, oral potassium citrate at a much lower dose has been used to treat low bone density with well-documented efficacy. The impact of citrate exposure during apheresis, either positive or negative, is important given that BMD is a significant risk factor for low trauma fracture. It is ultimately unknown what effect repeated apheresis has on skeletal health.

Added value of this study

We carried out a prospective, randomized, clinical trial testing the hypothesis that high frequency apheresis blood donation causes a decline in BMD. Forty-one donors completed the study and there was no change in bone mineral density among donors completing a median of 20 apheresis blood donations in the one year study period. Bone density did not change among members of the control group who did not undergo apheresis blood donation.

Implications of all the available evidence

Despite significant, repeated challenges to mineral homeostasis among apheresis blood donors, we conclude that current collection guidelines adequately protect the skeletal health of adult male, high frequency apheresis blood donors.

1. Introduction

Apheresis blood collections represent an increasing proportion of collected and transfused blood products in most parts of the world (Bialkowski et al., 2016). Apheresis involves the collection of specific blood components (e.g. platelets, red blood cells, plasma) by centrifuging whole blood in an extracorporeal circuit (Okafor et al., 2010). Anticoagulant is required to prevent coagulation in the circuit and is mixed with whole blood as it leaves the site of venipuncture. Citrate, the standard anticoagulant used during apheresis donation procedures (Hester et al., 1983; Lee and Arepally, 2012), exerts its anticoagulant effect by chelating ionized calcium (iCa) in donor plasma. Citrate is returned to apheresis donors intravenously as a mixture with blood components that have not been harvested. This return occurs every few minutes and elicits significant declines in ionized calcium (Hester et al., 1983; Bolan et al., 2001; Szymanski, 1978; Bolan and Leitman, 2002; Das et al., 2005). Apheresis donors subsequently experience alterations in circulating concentrations of parathyroid hormone (Bolan et al., 2001; Silberstein et al., 1986; Toffaletti et al., 1985; Mercan et al., 1997; Amrein et al., 2010; Bolan et al., 2003) vitamin D (Hiemstra et al., 2014), and markers of bone remodeling (Amrein et al., 2010; Bolan et al., 2003; Chen et al., 2009).

Evaluation of BMD among apheresis blood donors has yielded conflicting results. The prevalence of low BMD, defined as *Z*- and T-scores and adjusted for body mass index, average physical activity, and daily calcium intake, was higher for a group of apheresis donors (n = 102) as compared to a matched control group (Amrein et al., 2010). Another matched study of 20 apheresis and 20 whole blood donors, aged 55–70 and female, showed no difference in total hip or lumbar spine BMD (Boot et al., 2015). Both of these studies were cross-sectional and hence, purely correlative. Recent findings from a longitudinal Swedish study indicate that plasma apheresis does not increase the risk of fracture in blood donors (Grau et al., 2017); however, these findings apply to relatively low dose citrate AC exposure and relatively infrequent plasma apheresis donation.

The collection of platelets by apheresis is increasing in most parts of the world and confers citrate burden to the donor that is > 85% greater than for plasma apheresis (Evers and Taborski, 2016). Resolving the potential impact of the highest apheresis donation frequency, paired with the associated upper limit of citrate AC burden, represents a critical knowledge gap in ensuring adequate protections are in place to preserve the health of volunteer blood donors as it is their altruism that ensures the availability of life-saving blood products to patients in need.

2. Methods

2.1. Trial design

ALTRUYST (NCT02655055) was a randomized, longitudinal, controlled, single-center clinical trial designed to determine if repeated exposure to intravenous citrate through apheresis blood donation reduces BMD. Due to the fact that the vast majority of apheresis blood donors, particularly high frequency apheresis blood donors at the participating blood center, are Caucasian males, 18–65 year old males were selected as the population of interest. The *demographic* and *examination* data sets from NHANES were downloaded in April 2015 and used to estimate mean (1.055 g/cm^2) and standard deviation (0.135 g/cm^2) of total lumbar spine BMD for Caucasian males 18–65 years of age in the United States. These estimates were used to derive distributions of BMD using a multivariate, normal variable random sampling simulation (n = 10,000) with covariance for treatment included and change in lumbar spine BMD over a one year period exceeding the least significant change (LSC) at $\alpha = 0.05$ as the primary outcome. The LSC at the lumbar spine was used in final estimates of sample size which indicated that analysis of 20 apheresis and 15 control subjects was needed to achieve 80% power. Attrition between the two groups was expected to differ, with higher rates in the apheresis group anticipated. Therefore, a 2:1 block randomization scheme was employed with an overall target enrollment size of 45 (28 apheresis, 17 controls). The study was registered on ClinicalTrials.gov and approved by the Medical College of Wisconsin Institutional Review Board (IRB) with ceded review provided by the Marquette University IRB.

2.2. Participants

Male volunteer blood donors 18–65 years of age at enrollment were recruited at BloodCenter of Wisconsin using standard methods. Subjects with more than five lifetime apheresis blood donations were deliberately excluded to avoid any potential physiologic adaptation to citrate exposure. Subjects were also excluded if they had a metal prosthesis or previous fracture that could interfere with BMD measurement. Those weighing in excess of 300 lbs, with a previous fragility fracture, a previous lumbar spine fusion, cystic fibrosis, emphysema, celiac disease, Crohn's disease, current or past (> 1 month duration) use of corticosteroids or osteoporosis medications were also excluded. All subjects provided written informed consent prior to any study procedures.

2.3. Outcomes measurement

Dual energy X-ray absorptiometry (DXA) was performed at the lumbar spine, total hip, and total body using the GE iDXA™ with Encore version 11.40.004 software, trabecular bone score version 14.2, located at Marquette University. Two in vivo precision assessments of the individual technologist performing all BMD scans were performed in

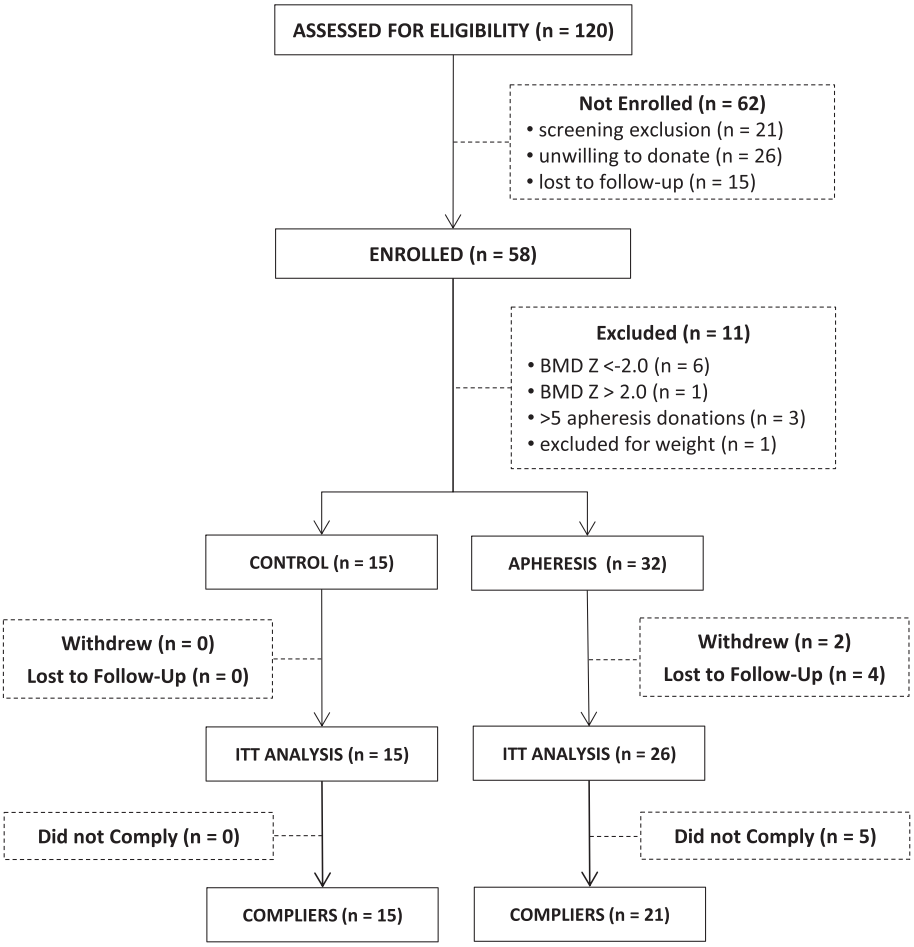

Fig. 1. Enrollment schematic showing recruitment, enrollment, ineligibility, and losses to follow-up for the ALTRUYST trial. ITT = intention to treat.

August 2015 per ISCD guidelines (Densitometry TISfC, 2007). In summary, 15 participants were scanned thrice, with repositioning in between scans. Participants in the precision assessment were male, between 18 and 65 years of age, generally healthy, and otherwise eligible for the research study. The technologist's LSC was 0.021 g/cm^2 at the lumbar spine and 0.021 g/cm^2 at the total hip.

2.4. Randomization and masking

A simple block randomization (block size of six) was used to allocate subjects to treatment or control groups. Research subjects and the research technologist were not blinded to treatment allocation. Paired sets of bone density scans were anonymized and blinded to both treatment status (apheresis or control) and time point (enrollment versus follow-up) before blinded clinician review (Dr. Joseph Shaker, Medical College of Wisconsin) using ISCD criteria (Densitometry TISfC, 2007).

2.5. Study procedures

An interviewer collected questionnaire data at enrollment to confirm eligibility and document race, ethnicity, family history of osteopenia/osteoporosis, family history of fracture, personal history of fracture, daily calcium intake, activity level, medication use, and other parameters that could alter baseline BMD (see Protocol, clinicaltrials.gov). Baseline DXA was performed prior to the first on-study blood donation. All subjects with a *Z*-score < -2.0 or > 2.0 at any measurement site were notified by letter and excluded from further participation. All participants received $50 for the completing the enrollment visit.

A peripheral blood sample was collected in a serum separator tube at the first on-study blood donation and processed within 30 min. Samples were subsequently frozen at −80 °C and tested (comprehensive metabolic panel and testosterone) within one year at ARUP Laboratories (Salt Lake City, UT). The duration of apheresis donation and volume of AC infused into the donor during the first of each procedure type was extracted from InfoVu, the online apheresis data logging software from TerumoBCT, and used to estimate total donation duration and volume. ALTRUYST investigators verified that component blood products derived during the study could be labeled as “voluntary units” (21 CFR 606.121(c)(8)(v)) per the FDA's Center for Biologics Evaluation and Research Office in February 2016. Hence, all blood components derived during ALTRUYST were available for transfusion.

Donors randomized to the apheresis arm were asked to make between 20 and 26 apheresis blood donations during the subsequent one year period and received $250 for doing so. Apheresis procedures were performed using the Trima Accel multi-component apheresis system (Burgstaler, 2006). Donors randomized to the control arm were asked to make zero or whole-blood-only donations during the subsequent one year period and received $100 for doing so. Participants were contacted one year hence (Gourlay et al., 2012) and returned to Marquette University for repeat questionnaire and BMD data acquisition as at enrollment. Subjects with a change in bone density that exceeded the technologist's LSC, or with *Z*-score < -2.0 or > 2.0 were reviewed by the medical monitor and notified by letter. Participants received $50 for completing the follow-up visit.

Table 1
Descriptive characteristics of ALTRUYST blood donors at enrollment.

	Apheresis	No apheresis	Total	*p*
n	26	15	41	–
Demographics				
Age (mean, SD)	42.6 (13.1)	45.9 (14.3)	43.8 (13.5)	0.46
Caucasian (n, %)	25 (96%)	15 (100%)	44 (98%)	–
Latino/Hispanic (n, %)	1 (4%)	0 (0%)	1 (2%)	–
Anthropometrics[a]				
Height (in.)	70.4 (2.4)	70.2 (3.2)	70.3 (2.7)	0.89
Weight (lbs)	203.2 (32.2)	191.7 (29.0)	199.0 (31.2)	0.25
Body composition	28.9 (4.7)	27.5 (5.0)	28.4 (4.8)	0.38
Laboratory data[a]				
Serum sodium (mmol/L)	143 (2.3)	142 (2.1)	143 (2.3)	0.24
Serum potassium (mmol/L)	4.5 (0.3)	4.7 (0.3)	4.6 (0.3)	0.38
Serum chloride (mmol/L)	101 (1.9)	100 (3.0)	101 (2.3)	0.37
Serum carbon dioxide (mmol/L)	22 (1.5)	21 (1.5)	22 (1.5)	0.19
Anion gap (mmol/L)	20 (2.2)	20 (1.8)	20 (2.0)	0.34
Serum urea nitrogen (mg/dL)	16 (3.7)	15 (5.3)	15 (4.2)	0.80
Serum creatinine (mg/dL)	1.00 (0.14)	0.99 (0.15)	1.00 (0.14)	0.83
Serum glucose (mg/dL)	97 (16)	101 (25)	98 (19)	0.66
Alkaline phosphatase (U/L)	67 (15)	75 (22)	70 (18)	0.33
Aspartate aminotransferase (U/L)	26 (8)	27 (4)	26 (7)	0.53
Alanine aminotransferase (U/L)	25 (12)	25 (7)	25 (10)	0.97
Serum calcium (mg/dL)	9.7 (0.4)	9.7 (0.5)	9.7 (0.4)	0.93
Serum inorganic phosphorous (mg/dL)	3.3 (0.5)	3.5 (0.4)	3.4 (0.5)	0.14
Serum total protein (g/dL)	7.2 (0.4)	7.4 (0.5)	7.3 (0.5)	0.39
Serum albumin (g/dL)	4.6 (0.3)	4.7 (0.3)	4.6 (0.3)	0.49
Serum total bilirubin (mg/dL)	0.6 (0.4)	0.6 (0.3)	0.6 (0.3)	0.95
Adult male testosterone (ng/dL)	520 (198)	515 (202)	518 (196)	0.94
Previous blood donations[b]				
Whole blood (n)	6	4	5	0.73
Apheresis (n)	3	3	3	0.49
Bone density[a]				
Lumbar spine (g/cm^2)	1.214 (0.130)	1.168 (0.120)	1.197 (0.127)	0.26
Total hip (g/cm^2)	1.133 (0.149)	1.026 (0.140)	1.094 (0.153)	0.03

[a] Mean (SD).
[b] Median.

2.6. Statistical analysis

The distributions of continuous and ordinal variables at baseline were compared using the *t*-test statistic and Fisher's exact test, respectively. The primary outcome was defined a priori as a decline in lumbar spine BMD that exceeded the technologist's LSC and secondary outcomes were change in total hip BMD and change in trabecular bone score. The intention to treat analysis included all subjects who completed follow-up. A per protocol analysis was also performed that included only those subjects who complied with the criteria of their randomization. Multivariable logistic regression was performed using the questionnaire, laboratory, baseline BMD data, and, treatment arm as predictors of change in bone density exceeding the LSC. Bilirubin, alkaline phosphatase, and anion gap were log transformed to achieve normal distributions. Automated stepwise backwards elimination was subsequently performed to identify significant predictors of both positive and negative response. All programming was developed and executed in R: a language and environment for statistical computing (Team RC, 2015). WB had full access to all study data and accepts final responsibility for the decision to submit for publication.

2.7. Role of the funding source

This work was supported by Marquette University and the BloodCenter Research Foundation. Neither entity had a role in study design; the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

3. Results

3.1. Enrollment

Among 120 volunteer blood donors assessed for eligibility, 58 enrolled in the study (Fig. 1). Seven enrollees were subsequently excluded for having a bone density *Z*-score < -2.0 or > 2.0 at enrollment. Three additional subjects were excluded for having previously undergone apheresis more than five times and one subject was excluded for weighing > 300 lbs. Ultimately, 32 subjects were randomized to the apheresis arm and 15 to the control arm.

Mean age at enrollment was 43.8 years (SD = 13.5) with one donor (2%) reporting African American race and another (2%) reporting Hispanic ethnicity (Table 1). Mean height ($p = 0.89$), weight ($p = 0.25$), and body mass index (BMI) ($p = 0.38$) were no different between study arms. Baseline laboratory parameters were similar between groups ($p = 0.14$–0.97), as were the number of previous whole blood (median = 5, $p = 0.73$) and apheresis (median = 3, $p = 0.49$) donations. Though lumbar spine bone density was no different between study arms ($p = 0.26$), bone density at the total hip was, on average, 0.107 g/cm^2 higher among those donors randomized to the apheresis arm ($p = 0.03$) (Table 1).

3.2. Follow-up

ALTRUYST donors made a total of 534 combined blood donations during the one year study period (Fig. 2). Approximately 20% of subjects from the apheresis arm were not available at follow-up: two subjects voluntarily withdrew from the study (6%) and four subjects were lost to follow-up (11%). All 15 (100%) donors randomized to the control arm complied with the protocol (three made zero whole blood donations, Fig. 2), whereas five (19%) apheresis donors did not achieve a minimum of 20 apheresis donations (Fig. 1, "Did not Comply"). The most common apheresis donation type was a double platelet donation with mean interval between donations of 17.8 days (Table 2). Donors in the apheresis arm experienced a median of 20 apheresis blood donations during the one year study period with the amount of citrate exposure by donation type ranging from 164 mL to 657 mL (Table 2). The duration of each donation ranged from just under 30 min to more than two hours in length.

3.3. High frequency apheresis for one year did not alter bone mineral density

Lumbar spine bone mineral density did not change among donors in the control arm after one year of participation (1.168 g/cm^2 at enrollment, mean change = -0.002 g/cm^2, 95%CI [-0.020, 0.016], $p = 0.16$), nor did it change among donors in the apheresis arm (1.214 g/cm^2 at enrollment, mean change = 0.007 g/cm^2, 95%CI [-0.005, 0.018], $p = 0.24$) (Fig. 3). Tests for differences in proportions of donors with change in BMD exceeding the least significant change (LSC) at the lumbar spine between the apheresis and control arms in either a positive [apheresis 8 (31%), control 4 (27%), $p = 0.78$] or negative direction [apheresis 4 (15%), control 5 (33%)] were statistically non-significant ($p = 0.18$) (Fig. 4). Performing the per protocol analysis (i.e. apheresis donors making ≥20 apheresis donations) did not meaningfully alter these results.

Change in mean BMD at the total hip was not statistically significant for control donors (1.026 g/cm^2 at enrollment, mean

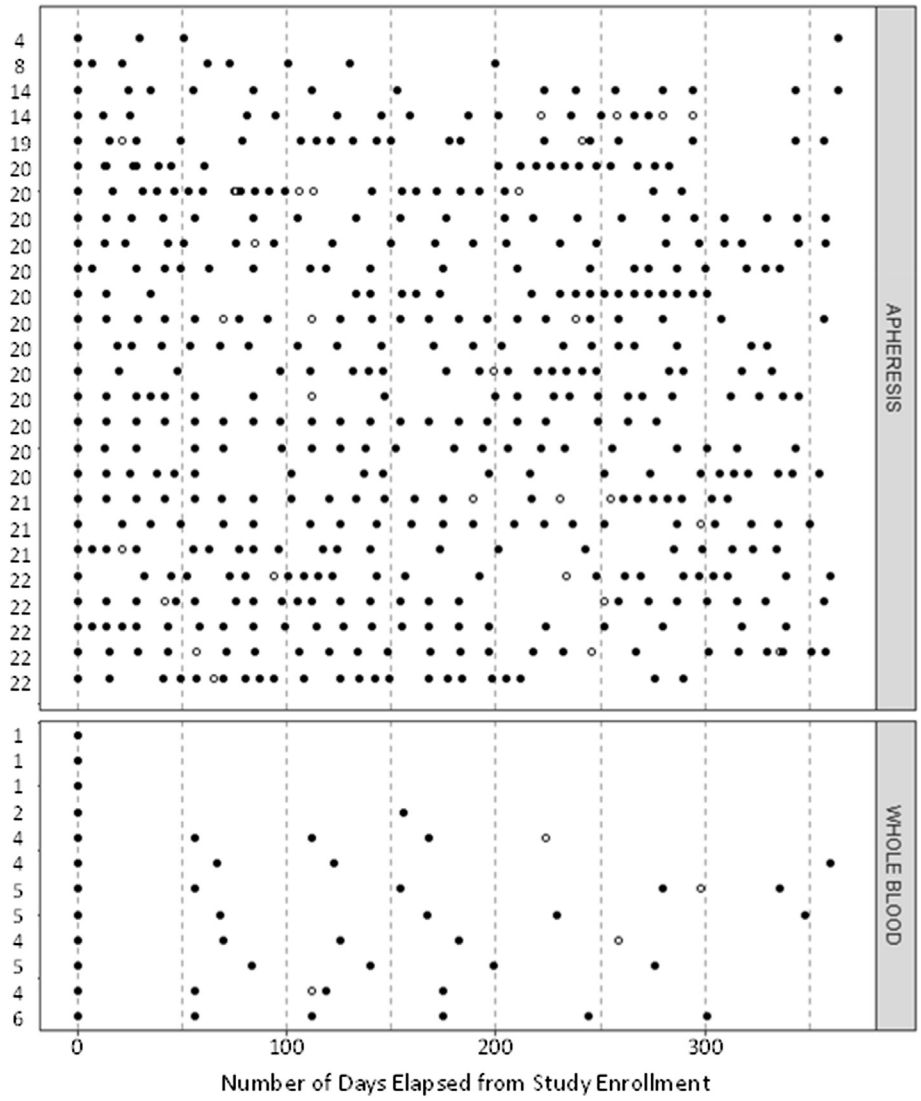

Fig. 2. Cleveland dot plot of donations (solid dots, n = 543) and deferrals (open dots, n = 38) from donors during the ALTRUYST study. The top panel shows donors randomized to apheresis and the bottom panel shows donors randomized to no apheresis (i.e. whole blood only). Each donor is represented as a row with the number of successful donations made during the study shown to the left.

Table 2
Apheresis collection and anticoagulant exposure characteristics for donors randomized to the treatment arm (high frequency apheresis) during the ALTRUYST trial.

		Single	Double	Triple
Collection information	Number of platelet apheresis donations	110	320	62
	Concurrent PLASMA COLLECTION n (%)	0 (0%)	4 (1%)	0 (0%)
	Mean (SD) collection time (min)	57.6 (21.8)	89.4 (23.3)	97.1 (24.9)
	Mean (SD) inter-donation interval (days)		17.8 (14.7)	
Anticoagulant exposure	Type of Anticoagulant		ACD-A[a]	
	Mean (SD) volume (mL) AC infused per procedure	299 (104)	469 (111)	498 (117)

[a] ACD-A = anticoagulant citrate dextrose solution, solution A (2.13% free citrate ion).

change = 0.002 g/cm^2, CI [−0.006, 0.009], p = 0.63) or apheresis donors (1.133 g/cm^2 at enrollment, 1.129 g/cm^2 at follow-up, mean change = −0.004 g/cm^2, CI [−0.10, 0.002], p = 0.16) (Fig. 3). Proportional increases [apheresis 0 (0%), control 1 (7%), p = 0.18] and decreases [apheresis 3 (12%), control 1 (14%)] were also not significantly different (p = 0.61) at the total hip (Fig. 4). Performing the per protocol analysis did not meaningfully alter these results. Multivariable logistic regression with change exceeding the LSC in both positive (gain in BMD) and negative (loss of BMD) directions using automated stepwise backwards elimination did not identify baseline covariates that were significantly associated with either outcome (Table 3).

Mean trabecular bone score was 1.388 (SD = 0.098) in the control group at enrollment and did not significantly change over the one year study period (1.406 (SD = 0.112) at follow-up, mean change = −0.003, 95%CI [−0.024–0.019], p = 0.79). Donors in the apheresis arm had a mean trabecular bone score at enrollment of 1.474 (SD = 0.105) and it did not change over the one year study period (1.475 (SD = 0.133) at follow-up, mean change = 0.001, 95%CI [−0.022, 0.024], p = 0.92).

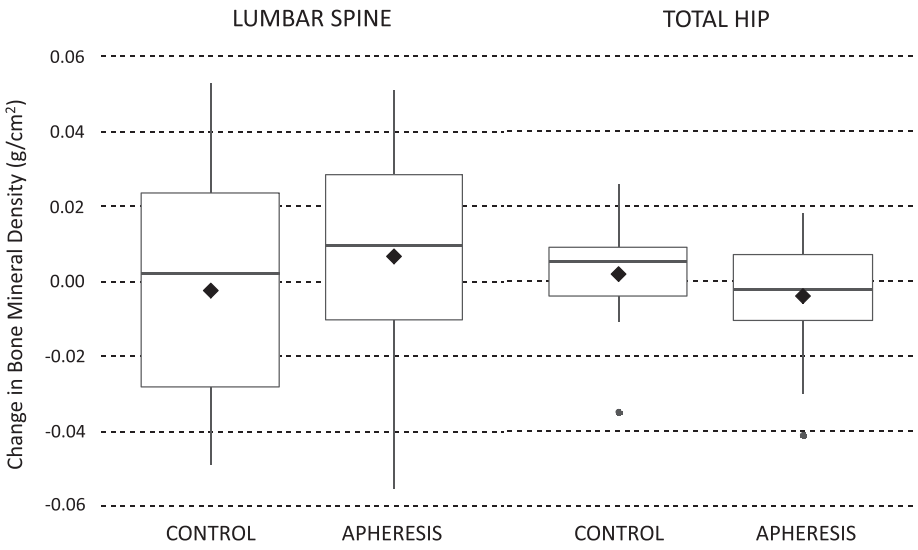

Fig. 3. Boxplots showing change in bone mineral density at the lumbar spine (left) and total hip (right) for control and apheresis donors in the ALTRUYST trial. Diamonds indicate mean values; median change is represented as the central horizontal bar within the interquartile range box.

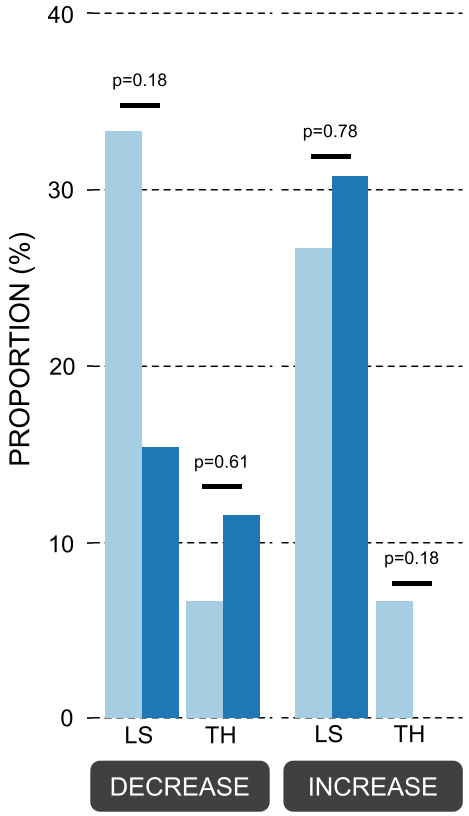

Fig. 4. Histograms showing mean change in lumbar spine BMD (top) and total hip BMD (bottom) in the per protocol analysis with control subjects (light blue) and apheresis donors (dark blue). *p* values represent test for proportions, both decrease and increase exceeding the least significant change, control versus treatment at the lumbar spine (LS) and total hip (TH). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

ALTRUYST was a prospective, longitudinal, randomized controlled trial evaluating the role of high frequency platelet apheresis blood donation on change in BMD and found no significant alterations to BMD at the lumbar spine, total hip, or significant change in trabecular bone score. Using individual blood donors as their own controls and performing longitudinal assessment of BMD represent differences from previous research studies that also tested the hypothesis that apheresis blood donation impacts bone density. Donors in the ALTRUYST trial almost exclusively donated platelets by apheresis (< 1% of donations involved concurrent plasma collections) and received an average of between 300 mL (single platelet apheresis) and 500 mL (triple collections) of AC per procedure with an average of 17 days between exposures. Though we are not able to directly compute dose in milliequivalents due to differences in apheresis machine settings, exposure in this study represents the upper limit of citrate AC exposure to volunteer platelet apheresis donor in the United States. We conclude that frequent platelet apheresis, and the associated exposure to citrate AC, does not induce changes in BMD exceeding 3% in a one year period. Furthermore, BMD of a control group randomly assigned to no apheresis remained unchanged during the study period, consistent with large studies of the male US population 18–65 years of age (Looker et al., 2010).

Previous studies demonstrated a higher prevalence of low BMD among apheresis donors when compared to non-blood donors (Amrein et al., 2010) or whole blood donor controls (Dettke et al., 2003), independent of donor gender or age. One study reported no difference among apheresis and whole blood donors (Boot et al., 2015). These conflicting results likely stem from the cross-sectional designs employed in these previous investigations. Analysis of > 140,000 Swedish blood donors over a 23 year period demonstrated no association between the risk of fracture and, mostly (94–98%), plasma apheresis (Grau et al., 2017). Plasma apheresis collections expose donors to a fraction of the AC that platelet apheresis donors receive (Evers and Taborski, 2016) and high frequency platelet apheresis donors were not present in the SCANDAT2 analysis and thus, the potential implications of high frequency platelet apheresis on bone density and fracture risk were not assessed.

Most ALTRUYST donors in the apheresis arm (21/26, 81%) achieved 20 or more apheresis donations during the one year study period and though no fractures occurred during ALTRUYST, trabecular bone score (TBS) was measured. TBS is an analytic tool that quantifies the extent of between-pixel differences in x-ray attenuation from DXA images approximating microarchitectural features of bone that are associated with increasing bone fragility and susceptibility to fracture (Jehle et al., 2013; Krueger et al., 2014). TBS did not change among ALTRUYST donors in the apheresis arm, or among donors in the control

Table 3
Results of exploratory multivariable logistic regression analysis with change in bone mineral density as the outcome [coefficient (*p* value)].

	Negative change		Positive change	
	Lumbar spine	Total hip	Lumbar spine	Total hip
Apheresis versus no apheresis	0.31 (0.08)	0.56 (0.74)	0.00 (0.99)	0.56 (0.74)
Age	−0.02 (0.07)	0.00 (0.94)	0.01 (0.45)	0.00 (0.94)
Risk factors = 1	−0.28 (0.11)	0.11 (0.92)	0.15 (0.54)	0.11 (0.92)
Risk factors = 2	−0.45 (0.07)	0.72 (0.76)	0.11 (0.67)	0.72 (0.76)
Risk factors = 3	−0.40 (0.14)	0.98 (0.71)	−0.46 (0.41)	0.98 (0.71)
Risk factors = 4	−3.62 (0.02)	0.74 (0.88)	2.51 (0.12)	0.74 (0.88)
Family risk factors = 1	0.15 (0.29)	−0.15 (0.94)	−0.11 (0.75)	−0.15 (0.94)
Family risk factors = 2	−0.13 (0.27)	−0.19 (0.91)	0.31 (0.41)	−0.19 (0.91)
Family risk factors = 3	−1.80 (0.04)	−0.46 (0.91)	0.76 (0.35)	−0.46 (0.91)
Health conditions = 1	0.71 (0.05)	0.59 (0.75)	−0.27 (0.44)	0.59 (0.75)
Health conditions = 2	2.60 (0.05)	0.75 (0.91)	−1.06 (0.41)	0.75 (0.91)
Medication use = 1	−1.13 (0.04)	−0.24 (0.90)	−0.96 (0.17)	−0.24 (0.90)
Diet = 3	0.21 (0.16)	0.65 (0.73)	−0.05 (0.22)	0.65 (0.73)
Diet = 4	0.30 (0.14)	0.61 (0.79)	−0.36 (0.39)	0.61 (0.79)
Diet = 5	0.91 (0.04)	−0.19 (0.90)	−1.56 (0.09)	−0.19 (0.90)
Diet = 6	1.52 (0.02)	0.55 (0.77)	−1.70 (0.10)	0.55 (0.77)
Diet = 7	4.18 (0.02)	1.33 (0.75)	−1.33 (0.26)	1.33 (0.75)
Body mass index	0.05 (0.05)	0.00 (0.98)	0.06 (0.17)	0.00 (0.98)
Baseline BMD	5.13 (0.02)	−1.59 (0.83)	−4.86 (0.10)	−1.59 (0.83)
Serum sodium	0.09 (0.14)	−0.27 (0.73)	−0.24 (0.18)	−0.27 (0.73)
Serum potassium	0.47 (0.10)	−0.73 (0.75)	−1.94 (0.09)	−0.73 (0.75)
Serum chloride	−0.18 (0.08)	0.14 (0.86)	0.11 (0.42)	0.14 (0.86)
Serum carbon dioxide	−0.28 (0.03)	0.17 (0.74)	0.21 (0.16)	0.17 (0.74)
Serum urea nitrogen	0.09 (0.03)	0.04 (0.77)	−0.10 (0.11)	0.04 (0.77)
Serum creatinine	−2.80 (0.04)	2.05 (0.83)	3.97 (0.11)	2.05 (0.83)
Serum glucose	−0.02 (0.04)	−0.01 (0.82)	−0.02 (0.17)	−0.01 (0.82)
Alkaline phosphatase	−0.01 (0.10)	0.01 (0.87)	0.00 (0.86)	0.01 (0.87)
Aspartate aminotransferase	0.00 (0.52)	0.04 (0.77)	0.00 (0.69)	0.04 (0.77)
Alanine aminotransferase	−0.08 (0.03)	0.01 (0.94)	0.07 (0.11)	0.01 (0.94)
Serum calcium	−0.93 (0.05)	0.13 (0.97)	1.28 (0.14)	0.13 (0.97)
Serum inorganic phosphorous	−0.36 (0.06)	0.12 (0.93)	−0.23 (0.33)	0.12 (0.93)
Serum total protein	1.21 (0.06)	0.12 (0.96)	−1.23 (0.20)	0.12 (0.96)
Serum albumin	−0.83 (0.11)	−0.19 (0.96)	2.25 (0.15)	−0.19 (0.96)
Serum total bilirubin	−0.30 (0.11)	0.19 (0.94)	−1.74 (0.07)	0.19 (0.94)
Adult male testosterone	0.00 (0.05)	0.00 (0.64)	0.00 (0.30)	0.00 (0.64)

arm. This finding is consistent with previous research showing that the magnitude of change in TBS is less than that of BMD area in the spine (Krieg et al., 2013; Popp et al., 2013; Senn et al., 2014). The finding that the upper limit of citrate AC exposure, both in terms of dose and frequency, failed to produce significant alterations to TBS among donors indicates that current apheresis collection guidelines do not likely alter fracture risk.

Several potential forms of bias and confounding were addressed in the design of ALTRUYST. Though mean BMD was higher in the ALTRUYST cohort relative to a gender- and age-matched sample of the US population, this is expected due to a well-known "healthy donor effect" where volunteer blood donors consistently present with health indices superior to population norms (Atsma and de Vegt, 2011). Furthermore, variability in the measure of central tendency among ALTRUYST donors indicates that individuals within the cohort were not contributing any outlier effects that could bias our assessment of the study's outcomes. This is, in part, due to the fact that ALTRUYST deliberately excluded individuals with BMD that fell outside of a 95% population-based estimate of mean BMD (i.e. ± 2 standard deviations of *Z*-score). Predisposition to diseases of bone and mineral metabolism were solicited from participants at enrollment, coded as an ordinal variable and assessed using multivariable logistic regression without achieving statistical significance for changes either in the positive or negative direction. The randomized design of this study ensured that the likelihood of being assigned to either group was equivalent. Nevertheless, confounding could occur if subjects experienced changes to other important determinants of BMD including physical activity, diet, and medication use. In addition to excluding subjects with known diseases of bone and mineral metabolism, subjects were deliberately excluded if they were taking medications known to impact BMD. Upon follow-up assessment, no changes in medication use were noted meaning that any confounding from medication use was absent. Physical activity did not differ between the two randomized groups, and, changes in physical activity sufficient to invoke changes in BMD over the one year interval did not occur. The self-report questionnaire was administered by the investigative team to avoid any non-response bias. These design features and observations indicate that the findings of ALTRUYST are extremely unlikely to have known bias or confounding that could have impacted the results of the study and though the potential for residual, unmeasured confounding can never be completely eliminated, the complete lack of any significant change in any outcome measured suggests that any such effect was minimal if present.

ALTRUYST deliberately studied male blood donors because they constitute the vast majority (approximately 80%) of the apheresis blood donor population at BloodCenter of Wisconsin, with 85% of higher frequency donors (defined as ≥15 apheresis donations within a one year period) also being male. Furthermore, the study recruited donors with no more than five lifetime apheresis donations so as to avoid any potential biological adaptation that may occur with repeated exposure to citrate AC. Though we report no change in bone mineral density among men, aged 18–65 years, experiencing high frequency apheresis over a one year period, we are unable to extrapolate these findings to women of any age. There remains the possibility that high frequency apheresis affects women differently than men, particularly during the peri-menopausal period when changes in serum estrogen have been correlated with large declines in BMD, with supplemental estrogen improving bone-related health outcomes (Kiel et al., 1987; Weiss et al., 1980). The scarcity of higher frequency female apheresis donors at the

blood center studied indicates that any exploration of the impact of high frequency apheresis on BMD among women would require a multi-center design Furthermore, the high prevalence of low BMD among women, especially that increases over the life course (Looker et al., 1995), indicates this would be an ideal group to evaluate the possible benefit of repeated alterations to PTH through apheresis (Bialkowski et al., 2016; Steddon and Cunningham, 2005) that resemble those of synthetic PTH treatments for osteopenia/osteoporosis with demonstrable improvement in BMD (Jehle et al., 2013).

Any change in BMD that exceeds the LSC is a clinically meaningful finding. Thus, another area for potential future investigation is the observation in ALTRUYST that high frequency apheresis induced clinically meaningful positive change in some donors and clinically meaningful negative change in others, particularly at the lumbar spine. The lumbar spine was deliberately selected as the site of the primary outcome measure because of the high surface area to volume ratio that could theoretically experience changes more quickly than other sites. Though ALTRUYST was not powered to detect such differences, this observation invokes the possibility that there may be individual differences in response to high frequency apheresis. Further research would be necessary to address this possibility.

5. Conclusions

Despite significant, repeated challenges to mineral homeostasis among apheresis blood donors through exposure to citrate AC, we conclude that current collection guidelines adequately protect the skeletal health of adult male, high frequency apheresis blood donors.

Authorship contributions

All authors were involved in the design of the study, the preparation of the manuscript, and approve the final version for publication. WB acquired all of the data. WB and CZ performed the analysis.

Declaration of interests

RDB is a site investigator for Novo-Nordisk, receives consulting fees from Amgen, Novo-Nordisk, Radius Health, and Ultragenyx, owns stock in Abbott Labs and Abbvie, receives authorship fees from McGraw Hill, and receives authorship royalties from UpToDate. All other authors have no competing interests.

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