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Blood donor obesity is associated with changes in red blood cell metabolism and susceptibility to hemolysis in cold storage and in response to osmotic and oxidative stress

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Abstract

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AUTHOR CONTRIBUTION

Tamir Kanias, Kelsey Hazegh, Grier P. Page, and Fang Fang conceived this study. Tamir Kanias, Kelsey Hazegh, and Angelo D'Alessandro wrote the manuscript. Tamir Kanias, Grier P. Page, Kelsey Hazegh, Fang Fang, Angelo D'Alessandro, Lorenzo Bertolone, Nareg Roubinian, and Larry Dumont designed, conducted and reviewed the statistical analysis. Johnson Q. Tran, Marcus O. Muench, and Rachael P. Jackman designed conducted and analyzed the human-to-mouse transfusion studies. Angelo D'Alessandro and Lorenzo Bertolone designed and performed the metabolomics analyses. Marjorie D. Bravo provided BMI data for Vitalant. All authors reviewed this manuscript before submission.

CONFLICT OF INTEREST

Though unrelated to the contents of the manuscript, A.D.A. is a founder of Omix Technologies Inc and Altis LLC, and a consultant for Hemanext Inc. All the other authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Background: Obesity is a global pandemic characterized by multiple comorbidities, including cardiovascular and metabolic diseases. The aim of this study was to define the associations between blood donor body mass index (BMI) and RBC measurements of metabolic stress and hemolysis.

Study Design and Methods: The associations between donor BMI (<25 kg/m^2, normal weight; 25-29.9 kg/m^2, overweight; and ≥30 kg/m^2, obese) and hemolysis (storage, osmotic, and oxidative; n = 18 donors) or posttransfusion recovery (n = 14 donors) in immunodeficient mice were determined in stored leukocyte-reduced RBC units. Further evaluations were conducted using the National Heart, Lung, and Blood Institute RBC-Omics blood donor databases of hemolysis (n = 13 317) and metabolomics (n = 203).

Results: Evaluations in 18 donors revealed that BMI was significantly ($P < 0.05$) and positively associated with storage and osmotic hemolysis. A BMI of 30 kg/m^2 or greater was also associated with lower posttransfusion recovery in mice 10 minutes after transfusion ($P = 0.026$). Multivariable linear regression analyses in RBC-Omics revealed that BMI was a significant modifier for all hemolysis measurements, explaining 4.5%, 4.2%, and 0.2% of the variance in osmotic, oxidative, and storage hemolysis, respectively. In this cohort, obesity was positively associated ($P < 0.001$) with plasma ferritin (inflammation marker). Metabolomic analyses on RBCs from obese donors (44.1 ± 5.1 kg/m^2) had altered membrane lipid composition, dysregulation of antioxidant pathways (eg, increased oxidized lipids, methionine sulfoxide, and xanthine), and dysregulation of nitric oxide metabolism, as compared to RBCs from nonobese (20.5 ± 1.0 kg/m^2) donors.

Conclusions: Obesity is associated with significant changes in RBC metabolism and increased susceptibility to hemolysis under routine storage of RBC units. The impact on transfusion efficacy warrants further evaluation.

Keywords

blood donors; BMI; hemolysis; obesity; red blood cells

1 | INTRODUCTION

Obesity, which is defined as a body mass index (BMI) of 30 kg/m^2 or greater, is a global pandemic that is associated with multiple comorbidities, including cardiovascular and metabolic diseases, chronic inflammation, oxidative stress, hyperlipidemia, cancer, and male hypogonadism.[1-3] According to the Centers for Disease Control and Prevention, the prevalence of obesity among adult Americans in 2017-2018 was 42.4%.[4] Obesity is also common among blood donors. A survey that evaluated over one million American blood donors between 2007 and 2008 revealed that over 29% (standardized prevalence) of all subjects were obese.[5] In support of this report, our evaluation of obesity in a large American blood service organization suggested that 36.2% of male donors had a BMI of ≥30 kg/m^2. The same analyses have identified extraordinary high prevalence of obesity (50.8%) among male donors who received testosterone replacement therapy (TRT).[6] We previously reported that testosterone and TRT are modifiers of red blood cell (RBC) susceptibility to hemolysis,

[6-8] and investigations of the mechanisms of this phenomenon led us to the discovery of obesity-related changes in RBC biology, metabolism, and hemolysis.

Obesity may alter RBC metabolism and susceptibility to hemolysis via diverse pathways. For example, RBCs from obese subjects with metabolic syndrome exhibited 25% reduction in deformability and increased membrane rigidity as compared with RBCs from nonobese subjects.[9] Another study that investigated the impact of a high-fat diet, a hallmark of obesity and atherosclerosis, on murine RBC function found increased formation of reactive oxygen species; expression of proinflammatory and proeryptotic (early RBC death) markers, including chemokine release via the Duffy antigen receptor and membrane phosphatidylserine exposure, which promotes macrophage-mediated phagocytosis.[10] Similarly, RBCs from obese subjects exhibited reduced expression of CD47, a major antiphagocytosis antigen.[11] Obesity has also been correlated with increased RBC aggregation that could compromise blood flow and tissue oxygenation,[12,13] and with increased RBC distribution width, which was correlated with markers of inflammation.[14] In light of these observations, we hypothesized that comorbidities associated with obesity (eg, oxidative stress, chronic inflammation, hypertension, atherosclerosis, hyperlipemia) alter RBC biology and capacity to survive cold storage and transfusion.

In this study, we conducted retrospective analyses on two cohorts of blood donors with existing data of blood donor BMI and measurements of the effect of storage on RBC concentrates, including spontaneous and stress-induced hemolysis, and RBC metabolism. The first cohort consisted of nine patients undergoing TRT and nine matched controls (Vitalant Denver) that further provided data regarding the association between donor BMI and RBC posttransfusion recovery in immunodeficient mice. The second cohort included 13 317 blood donors who participated in the National Heart, Lung, and Blood Institute's (NHLBI) RBC-Omics study,[15] which was aimed at identifying genetic and biologic modifiers of hemolysis in stored RBCs. In both cohorts, we quantified the associations between blood donor BMI and in vitro measurements of RBC stress, including spontaneous hemolysis in response to cold storage, osmotic fragility, and oxidative hemolysis induced by 2′-azobis(2-amidinopropane) dihydrochloride (AAPH). The osmotic and oxidative stress assays had been proven highly reproducible[16] and successful (RBC-Omics) in disclosing genetic and metabolic factors that regulate RBC membrane integrity, volume, and antioxidant capacity.[17-19] We further used the RBC-Omics database of RBC metabolomics in a subset of recalled donors with low and high BMI scores. This analysis provided additional insights into the mechanisms that could explain the observed changes in RBC predisposition to osmotic and oxidative hemolysis in blood donors with obesity.

2 | MATERIALS AND METHODS

2.1 | Human subjects

This study was conducted under regulations applicable to all human subject research supported by federal agencies, including institutional review board approval from each participating site. The data presented in this study were obtained from two databases of blood donors with available demographic and RBC storage outcomes (eg, hemolysis, metabolomics). The first database included data from 18 eligible blood donors (9 who

received TRT and 9 matched controls; 16 men and 2 women) who presented at Vitalant Denver in 2019. Deidentified information including height, weight, and TRT status was collected from the consented donors through eProgesa Database. The second database was from 13 317 blood donors who participated in the NHLBI Recipient Epidemiology Donor Evaluation Study III (REDS-III) RBC-Omics study between 2013 and 2015.[15,19,20] This database provided demographic information, including donor BMI, age, sex, race/ethnicity, plasma ferritin, RBC hematologic indices, spontaneous (cold storage) and stress hemolysis scores, and metabolomics data from a subset of blood donors, who were recalled based upon hemolysis scores.[16,17]

2.2 | RBC storage and hemolysis assays

Whole blood donated by the first cohort (Vitalant Denver) was leukoreduced (LR), and the RBC concentrates were stored (1-6°C, 42 days) in additive solution-3 in accordance with Vitalant's standard operating procedures. In RBC-Omics, whole blood units donated by donors who gave consent were leukocyte reduced and the packed RBCs were mixed into additive solution-1 or −3 according to each participating blood center standard operating procedures. A representative portion (10-15 mL) of RBCs from each LR-RBC unit was then sterile transferred into a customized transfer bag (Haemonetics, Braintree, Massachusetts), which was stored (1-6°C) for 42 days. Metabolomics data were obtained from recalled blood donors[16] who donated a full LR-RBC unit, which was stored in additive solution-1 or −3 for 42 days and tested for hemolysis (Days 10, 23, and 42) and aliquots frozen (at −80°C). Detailed information about RBC-Omics including validation studies is reported in detail elsewhere.[21] Stored RBCs were evaluated for spontaneous (cold storage) and two stress hemolysis assays including osmotic fragility using a modified pink test,[8,22] and oxidative hemolysis using AAPH as described before[8] and detailed in the Supporting Information.

2.3 | Determination of RBC posttransfusion recovery in NSG mice

Animal research was conducted with approval and over-sight of the Institutional Animal Care and Use Committee at Covance Laboratories, Inc. (San Carlos, CA) under Animal Welfare Assurance A3367-01. The data reported in this study were from 14 blood donors (Vitalant cohort), for whom we quantified RBC posttransfusion recovery in immunodeficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice.[23] Further information is available in our recent publication[24] and the Supporting Information.

2.4 | Effect of donor BMI on RBC metabolome

RBC-Omics has a database of 203 recalled subjects with metabolomics data on LR-RBC units, which were stored for 42 days and tested at three time points (Days 10, 23, and 42).[16,25,26] These subjects were selected based upon their RBC hemolysis scores representing the lowest (5th percentile) or highest (95th) susceptibility to storage, osmotic, or oxidative stress. From this database, we selected subjects with the lowest and highest BMI (15 per group) and performed an unsupervised hierarchical clustering analysis[27] of metabolomics at storage days 10, 23, and 42 for the low and high BMI groups (20.5 ± 1.0 kg/m^2 vs 44.1 ± 5.1 kg/m^2, respectively). Metabolomics data were analyzed by MetaboAnalyst[27] and normalized before further processing (mean-centered and divided by the standard deviation of each variable) prior to one-way analysis of variance (ANOVA). The *P* values from these analyses

are reported in Table S1. Testing for statistical differences in metabolites between the two BMI groups were performed by nonparametric Wilcoxon tests, as some variables were not normally distributed. Metabolomics graphs and statistical analyses were prepared with computer software (Prism 8.0; GraphPad Software, Inc, La Jolla, California).

2.5 | Statistical analysis

The correlation between BMI and hemolysis was determined either by using BMI as a continuous variable or by comparing the hemolysis data among three clinically defined BMI categories, including 18.5-24.9 kg/m^2 (normal weight), 25-29.9 kg/m^2 (overweight), and 30 kg/m^2 or greater (obese). One or two-way repeated-measures ANOVA was applied to compare the means across the three BMI groups for each measure with Prism 8.0 software. For variables that were not normally distributed (storage hemolysis and ferritin), a nonparametric Kruskal-Wallis test was used. The relationship between BMI and each hemolysis measurement or ferritin was assessed using regression analysis with smoothing splines with computer software (R package ggplot2, version 3.2.1; R Foundation, Vienna, Austria).[28] The smoothed splines were estimated by the method “gam” (generalized additive model; version 1.8-28), which uses a back-fitting algorithm to combine different smoothing methods. For each BMI value, a predicted mean value for each hemolysis measurement and ferritin was plotted on the fitted lines with shaded areas indicating the 95% confidence intervals for the standard error of the mean (SEM).

2.6 | Multivariable linear regression

To assess and account for potential confounding factors, we applied a multivariable linear regression to quantify the effect of BMI on each hemolysis measurement adjusted for race/ethnicity, age, donation history (number of donations within the past two years), sex, hormone intake, and ferritin levels. Interactions such as “BMI × Hormone Intake” were included in linear models when appropriate. Adjustment for RBC component collection or manufacturing differences was conducted before the analysis as described before.[19,21] In the multivariate analysis, we considered *P* values less than .001 as the statistically significant threshold to account for multiple statistical tests, consistent with our previous work.[19] To evaluate the effect of each covariate in the model, we initially determined the goodness-of-fit measure (R^2) for each hemolysis measurement. The sum of squares of each covariate divided by sum of squares total gave the proportion of variance explained (PVE) in each model. The r^2 for each variable was calculated by PVE × R^2.

3 | RESULTS

3.1 | RBCs from donors with BMI of 30 kg/m^2 or greater exhibit increased spontaneous and osmotic hemolysis and reduced posttransfusion recovery

We initially investigated the association between BMI and hemolysis, including posttransfusion recovery in NSG mice from 18 blood donors (Vitalant’s cohort), whose demographic determinants are summarized in Table 1. We combined data from all 18 subjects in this analysis, as testing for interactions between BMI, hemolysis, and TRT status (Table S2) revealed no significant interactions between TRT and BMI. As demonstrated in Figure 1A and B, donor BMI was positively associated with storage (Pearson r = 0.543; *P* =

0.0187) and osmotic hemolysis (Pearson r = 0.613; $P = 0.007$). Specifically, both measures of hemolysis were higher in obese donors (BMI ≥30 kg/m^2) as compared with normal weight or overweight donors, and the differences among the three BMI groups were evident at the first week of cold storage for osmotic hemolysis, and from Week 2 onward for storage hemolysis. Furthermore, evaluation of the increase in hemolysis over time suggested that the rate of degradation, in terms of storage and osmotic hemolysis, was higher in RBCs from obese subjects. Statistically significant differences ($P < 0.05$) between obese and nonobese donors were noted in Weeks 5 and 6 (Figure 1A,B). In this cohort, no significant differences were observed among the BMI groups with regard to oxidative hemolysis (Figure 1C).

With regard to posttransfusion recovery in NSG mice (Figure 1D and Table S3), stored RBCs from donors with obesity had lower posttransfusion recovery compared with RBCs from nonobese donors shortly after (10 minutes; $P = 0.026$ between BMI categories of 25-29.9 and ≥30 kg/m^2) and at 2 hours after transfusion (not significant), whereas the differences among the groups become smaller at 4 hours, 8 hours, and 24 hours after transfusion. Of note, average RBC posttransfusion recovery was consistently higher (not statistically significant) in subjects with a BMI less than 25 kg/m^2 (normal weight) as compared with RBCs from donors with BMI of 30 kg/m^2 or greater.

3.2 | Interactions between donor BMI and hemolysis or ferritin in RBC-Omics

We further evaluated the impact of donor BMI and obesity on RBC susceptibility to hemolysis using the REDS-III RBC-Omics database of hemolysis in 13 317 blood donors.[15,19] The distribution of BMI (mean and SD) in each racial/ethnic group and between the sexes is summarized in Table 2. Donor race/ethnicity was a significant ($P < 0.001$) determinant of BMI in male and female donors. For example, average BMI was lowest in Asian female donors (24.9 ± 4.3 kg/m^2) and highest in African American female donors (30.2 ± 6.9 kg/m^2). The average BMI in all racial/ethnic groups was above 25 kg/m^2 (overweight and obese categories).

A regression analysis that evaluated the correlation between donor BMI and hemolysis or ferritin in RBC-Omics (Figure 2 and Figure S1) revealed sex-specific differences in each measured outcome. In women, BMI was positively associated with the three hemolysis measurements (Figure 2A-D) and plasma ferritin (Figure 2E, F). In men, mean storage hemolysis peaked around BMI of 35 kg/m^2 and plateaued thereafter (Figure 2A). The increase in osmotic hemolysis plateaued around the overweight range (BMI of 25-29.9 kg/m^2), after which a second peak was observed around 35 kg/m^2 (Figure 2B). We previously reported that donor age and frequent blood donations were negatively correlated with AAPH-induced oxidative hemolysis and donor plasma ferritin.[19,29] We therefore evaluated the relationship between BMI and these two variables in all donors (Figure 2C, E) and in first-time donors (Figure 2D, F). As previously reported,[29] first-time donors had higher oxidative hemolysis and ferritin levels as compared to repeat donors. Despite these differences, we found similar associations between BMI and oxidative hemolysis or ferritin, independent of donation frequency with the exception of oxidative hemolysis in first-time male donors (Figure 2D), in which increased hemolysis was observed in donors within the lower BMI extreme. As reported in Table 3, these donors were likely to be underweight

(BMI <18.5 kg/m^2). Overall, the effect of donor BMI on the hemolysis measurements or ferritin was more pronounced in male donors than in female donors (Table S4).

Table 3 summarizes the distribution of each hemolysis measure, donor hemoglobin, and plasma ferritin at selected BMI bins. In this analysis, BMI in male and female donors was positively and significantly ($P < 0.001$) associated with all hemolysis measurements and with plasma ferritin. In both sexes, obesity was not associated with significant changes in donor hemoglobin, although we observed a trend toward lower hemoglobin in men at a BMI of 50 kg/m^2 or greater.

3.3 | Multivariate analysis of RBC-Omics database identifies BMI as a significant modifier of osmotic and oxidative hemolysis

A multivariate linear regression model that adjusted for donor sex, age, race/ethnicity, donation history (number of donations within the past two years), sex hormone intake, and ferritin (Table 4) revealed that BMI was significantly ($P < 0.001$) associated with osmotic and oxidative hemolysis. In this model (based on the r^2), donor BMI explained 4.5% of the variability in osmotic hemolysis, 4.2% of the variability in oxidative hemolysis, and only 0.2% of the variability in storage hemolysis. Donor sex explained 5.4% and 0.8%, and 1.0% of the variations in osmotic, oxidative, and storage hemolysis, respectively. Donor race/ethnicity was a major modifier for osmotic hemolysis ($r^2 = 4.2\%$). Donor age was a significant modifier of oxidative hemolysis ($r^2 = 4.2\%$).

3.4 | Metabolomics analyses of subjects with extreme BMIs in the REDS-III RBC-Omics recalled donor cohort

The demographic determinants and hemolysis scores of the recalled donors are summarized in Table S5, which reported significantly ($P < 0.001$) higher levels of oxidative hemolysis and ferritin levels in the high-BMI group as compared with the low-BMI group. Metabolomic analysis (Figure 3A) suggested that BMI had a significant impact on RBC metabolism. Specifically, we noted significant alterations in fatty acid and amino acid composition and levels in RBCs from the high BMI group (Figure 3B). These observations were accompanied by dysregulation of glutathionylated-adducts of oxidized components (oxidized fatty acids, sugars and gamma-glutamyl-cycle metabolites) in the high-BMI group. Specifically, RBCs from donors in the high-BMI group were characterized by higher levels of lysophosphoinositol lipids (18:0, 18:2, 20:4) and lysophosphatidylserines (18:1; Figure 4A). The high-BMI group was also characterized by increased levels of short-chain fatty acid products of lipid peroxidation (6:0, 7:0; storage Days 23 and 42) and short-chain acyl-carnitines (Figure 4B). Additionally, several glutathionylated lipid peroxidation products were increased (not significant) in stored RBCs from these donors on Day 10 and throughout storage (Figure 4C).

Additional soluble markers of oxidative stress to the hydrophilic component of the metabolome, namely, methionine sulfoxide (a product of methionine oxidation[26]) and xanthine (product of oxidant stress-dependent purine deamination)[30] were increased in stored RBCs from donors with high BMI throughout storage (Figure 4D), in which significant ($P < 0.05$) differences in methionine sulfoxide levels were observed on days 10

and 42. Differences between the two BMI groups were also noted in the rates of conversion of arginine to citrulline, a reaction that produces nitric oxide. Arginine levels were significantly higher in the high-BMI group, whereas citrulline levels were higher in the low-BMI group on storage Days 23 and 42 (Figure 4E). In keeping with the apparent increase of oxidative stress in stored RBCs from donors with high BMI, increased levels of tryptophan and its oxidation product kynurenine were observed throughout storage (Figure 4F). Finally, other amino acids involved in ketogenesis and gluconeogenesis (eg, leucine and valine) were higher in RBCs from donors with high BMI (Figure 4G); however, these differences did not reach statistical significance, except for valine at storage Day 10.

4 | DISCUSSION

This study identified BMI and obesity as modifiers of in vitro measurements of hemolysis in two independent cohorts of blood donors. The outcomes provide new knowledge regarding obesity-linked changes in RBC biology, metabolism, susceptibility to osmotic and oxidative stress, and recovery following routine blood banking storage of LR-RBC units. The observation of increased storage hemolysis in high-BMI donors is in agreement with a comparable study from Australia.[31] In our view, blood donors provided an excellent model for studying the interactions between obesity and hemolysis, as this population is considered healthier than the general population[32] and is routinely screened for potential confounders (eg, high blood pressure, hemolytic diseases, intake of blood thinners) that could impact our observations. Given the growing rates of obesity in the general population and among blood donors, possible outcomes on the quality of blood products and blood supply warrant further investigation.

Our evaluation of hemolysis in RBC units from the Vitalant cohort supported the hypothesis that donor BMI modulates RBC susceptibility to hemolysis during cold storage and in response to osmotic stress. Although it can be argued that these *in vitro* measurements of hemolysis may not reflect clinical outcomes in transfused patients, we found that increased storage and osmotic hemolysis, as observed in the group with BMI 30 kg/m^2 or greater, was associated with reduced posttransfusion recovery in NSG mice 10 minutes after transfusion. This observation suggests that prolonged (39-42 days) storage of RBCs from donors with BMI of 30 kg/m^2 or greater may be associated with increased clearance of transfused RBCs shortly after transfusion. It should be noted that this observation has several limitations, including a small sample size (n = 14), of which only three donors had a BMI of less than 25 kg/m^2 (normal weight) and that survival in the mouse circulation may not simulate outcomes in transfused humans. Despite these limitations, we observed a trend towards lower posttransfusion recovery in RBCs from donors with obesity, which was remarkable given the relatively small variation in this cohort's BMI scores (28.2 ± 3.8 kg/m^2) and the fact that only one subject had a BMI greater than 35 kg/m^2, which is defined as severely obese.

Characterization of donor demographics associated with BMI in 13 317 blood donors from RBC-Omics revealed sex and racial/ethnic differences in the prevalence of obesity. The highest BMI values were observed in men and in African American donors, whereas lower BMI values were observed among women and in Asian Americans. This variation in BMI is likely the combination of physiological differences between the sexes and among ethnic

groups, as well as behavioral and socioeconomic factors that are associated with obesity (eg, nutrition habits, lower income).[33]

Our previous analyses in the RBC-Omics cohort had identified sex, age, sex hormone intake, and donation frequency as significant modifiers of RBC predisposition to hemolysis in response to cold storage or under osmotic and oxidative stress.[19,29,34] The present analyses supported our findings from the Vitalant cohort, suggesting a role for BMI and obesity as additional significant modifiers of RBC characteristics related to osmotic fragility and antioxidant capacity. Although these associations were observed in both sexes, BMI-mediated changes in hemolysis or donor ferritin were greater in men than in women. This suggests that underlying conditions such as obesity further contribute to sex-specific differences in RBC storage stability and to outcomes in hemolytic disease.[35] Of note, the association curves between BMI and hemolysis in male donors were not linear in all cases. For example, oxidative hemolysis in stored RBCs from first-time male donors was highest at BMIs less than 18.5 kg/m^2 (underweight) and 35 kg/m^2 or greater. Increased RBC susceptibility to oxidative hemolysis, as observed at BMIs less than 18.5 kg/m^2, can be partially explained by the younger age of donors in this group (median age, 26.5 years), which have been associated with higher oxidative hemolysis as compared with older donors.[19] Since RBC-Omics' database only included a small number of underweight donors (n = 28), we were unable to evaluate the impact of this condition on RBC predisposition to hemolysis. The correlation between BMI and ferritin including the higher levels of ferritin observed in donors with obesity may be indicative of inflammation rather than a marker of iron stores.[36] However, the reported ferritin levels in all donors (Table 3) were within the adult normal limit of less than 300 ng/mL.

On the basis of the multivariate regression analysis and percentage of variance explained, donor BMI accounted for about 4.2% of the variability in oxidative hemolysis. Obesity has been associated with compromised antioxidant enzyme activity (eg, cytochrome P450) and vitamin deficiency,[37] which may explain the greater susceptibility of RBCs from donors with high BMIs to oxidative stress. BMI also accounted for about 4.5% of the variability in osmotic hemolysis. The mechanisms that increase RBC susceptibility to osmotic and oxidative stress in donors with obesity can be partially explained by the metabolomics analyses, which associated obesity with alterations in membrane lipid composition and with dysregulation of antioxidant pathways characterized by increased levels of oxidized lipids, methionine sulfoxide, and xanthine. RBC lipid composition profile in donors with obesity was suggestive of lipidemia, as the RBC lipidome provides a reliable indication of the systemic lipidome.[38-40] Additional metabolic markers of RBC membrane lipid remodeling in donors with obesity were short-chain fatty acid (6:0, 7:0) and short-chain acyl-carnitines. Since the latter is in equilibrium with coenzyme A–conjugated fatty acids,[41] it is interesting to speculate that RBC lipid remodeling in obesity is induced by lipolysis, oxidation (increased short-chain fatty acids), and alteration of the lysophopsholipid-repair pathway or the Lands' cycle.[42] Of note, incubation of human RBCs in lipemic plasma from blood donors has been shown to induce hemolysis.[43] Similarly, lipemia has been associated with increased susceptibility to osmotic fragility in canine RBCs.[44]

The conversion of arginine to citrulline by the nicotinamide adenine dinucleotide phosphate-dependent nitric oxide synthase is essential for the regulation of vascular tone via the release of nitric oxide.[45] Accumulation of arginine and depletion of citrulline, as observed in RBCs from donors with obesity, may compromise RBC-mediated vascular tone regulation and vasodilation.[46,47] In keeping with the apparent increase of oxidative stress in stored RBCs from donors with obesity, increased levels of tryptophan and its oxidation product kynurenine were observed throughout storage, which is relevant in the light of the role of these metabolites in immunomodulation and inflammation.[48,49] Finally, other amino acids involved in ketogenesis and gluconeogenesis (eg, leucine and valine) were higher in RBCs from subjects with obesity. This observation is relevant in that the circulating levels of these and other branched-chain amino acids have been proposed as early markers of obesity-associated diabetes.[50]

This study had several limitations: the male-to-female ratio in the first cohort was 16:2, and this discrepancy may have impacted our observations given the sex differences in hemolysis (eg, Figure 2) and the weaker associations between BMI and hemolysis in female donors (Table S4). The metabolomics analyses were performed on groups of donors with the most extreme BMIs enrolled in this study, and, as such, results may not necessarily translate for donors with obesity with intermediate BMIs. In addition, a complete metabolomics data (ie, testing Days 10, 23, and 42) was not available for all subjects as per RBC-Omics study design. Despite this limitation, each group (low and high BMI) was still composed of 15 subjects per time point falling within the categories described in the study. Finally, the multivariate analysis has identified other significant modifiers (sex, age, race/ethnicity) of hemolysis.

In conclusion, this study quantified and characterized the associations between blood donor BMI and hemolysis in two cohorts of American blood donors. We demonstrated that obesity is associated with significant changes in RBC metabolism and increased susceptibility to spontaneous or stress-induced hemolysis under routine storage of LR-RBC concentrates. These observations may have clinical implications in the form of low-grade hemolysis in subjects with obesity that may contribute to the development of vascular dysfunction in cardiovascular and metabolic diseases. The relevance of these observations to RBC storage and transfusion efficacy is not clear and warrants further evaluations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AAPH	2′-azobis(2-amidinopropane) dihydrochloride
ANOVA	analysis of variance
BMI	body mass index
LR	leukoreduced
NSG	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ
PVE	proportion of variance explained
REDS-III	Recipient Epidemiology Donor Evaluation Study III
TRT	testosterone replacement therapy

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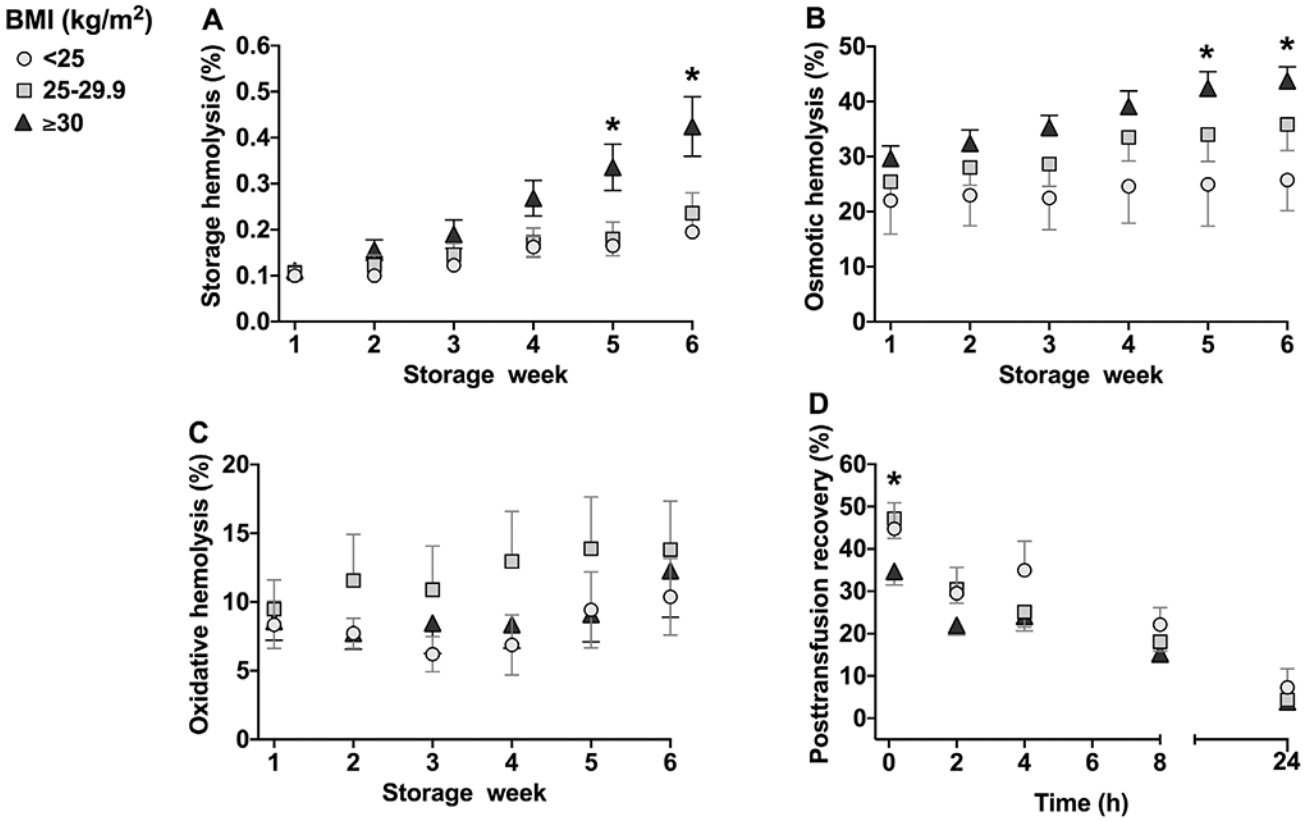

FIGURE 1.

Blood donor BMI is associated with increased hemolysis during cold storage of RBCs and with reduced posttransfusion recovery in NSG mice. Leukoreduced RBC units from 18 donors were stored for 6 weeks and tested weekly as described under methods. Hemolysis measurements were evaluated for each BMI group (<25 kg/m^2, normal weight, n = 4; 25-29.9 kg/m^2, overweight, n = 7; ≥30 kg/m^2, obese, n = 7). A, Percent storage hemolysis. B, Percent osmotic hemolysis. C, Percent AAPH-induced oxidative hemolysis. D, Posttransfusion recovery of 6-week-old leukoreduced RBC transfused into NSG mice. N = 14 of which n = 3 for BMI <25 kg/m^2, n = 6 for BMI 25-29.9 kg/m^2, and n = 5 for BMI ≥30 kg/m^2. Data are represented as mean ± SEM. Asterisks denote significance ($P < 0.05$, repeated measures two-way ANOVA with Bonferroni's multicomparison test) of differences between nonobese (<25 or 25-29.9 kg/m^2) and obese samples (A); <25 vs ≥30 kg/m^2 (B); and between 25-29.9 and BMI ≥30 kg/m^2 (D)

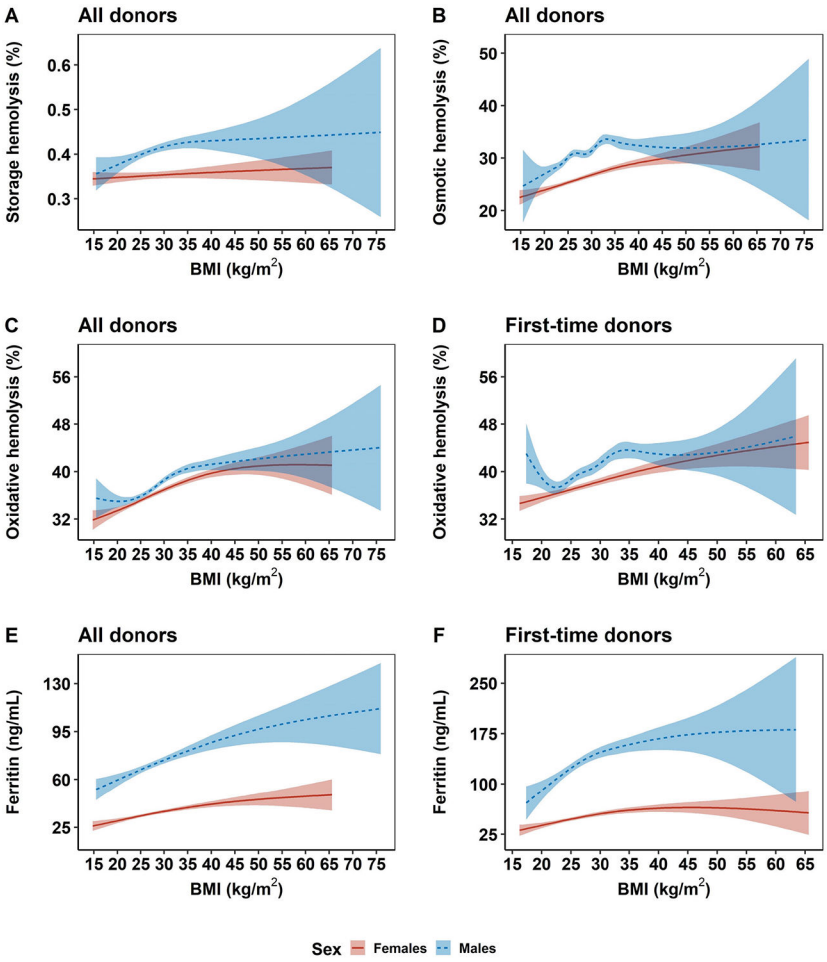

FIGURE 2.

Distribution of storage hemolysis, stress-induced hemolysis, or plasma ferritin by blood donor BMI and sex. A-D, Leukoreduced RBCs from male and female donors who participated in NHLBI's RBC-Omics study (n = 13 197) were stored for 39 to 42 days and tested for storage- or stress-induced hemolysis. A, Percent storage hemolysis by BMI. B, Percent osmotic hemolysis by BMI. C and D, percent oxidative hemolysis by BMI in all donors (C) and first-time donors (D). E and F, Plasma ferritin levels (ng/mL) by BMI in all donors (E) and first-time donors (F). The shaded regions in all plots represent the 95% confidence intervals for the SEM

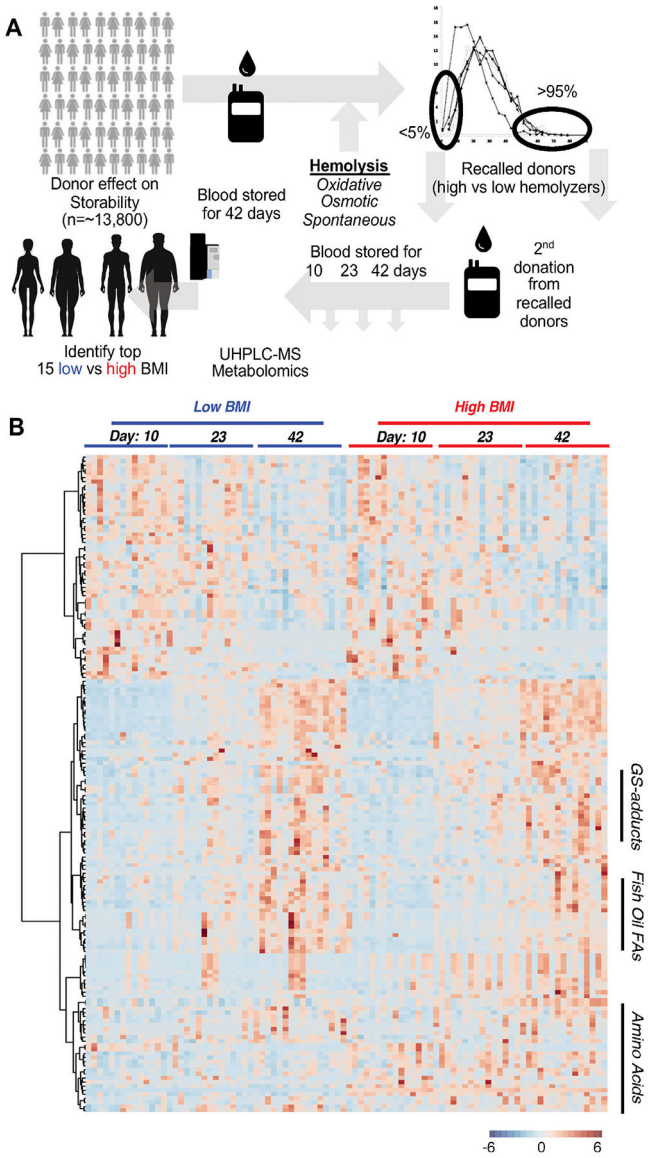

FIGURE 3.

Metabolomics analyses of REDS III RBC-Omics recalled donors as a function of BMI. Blood donors were classified on the basis of BMI (20.5 ± 1.0 kg/m^2 defined as low BMI vs 44.1 ± 5.1 kg/m^2 defined as high BMI) before analysis of previously acquired metabolomics data from RBC units stored for 10, 23, and 42 days (A). The heat map in B shows the metabolites significant by ANOVA. A subgroup of metabolites emerged as distinctive between low- and high-BMI subjects, specifically metabolites involved in glutathione homeostasis (and glutathione adducts), lipid metabolism (especially fish oil and free fatty acids), and amino acids (B)

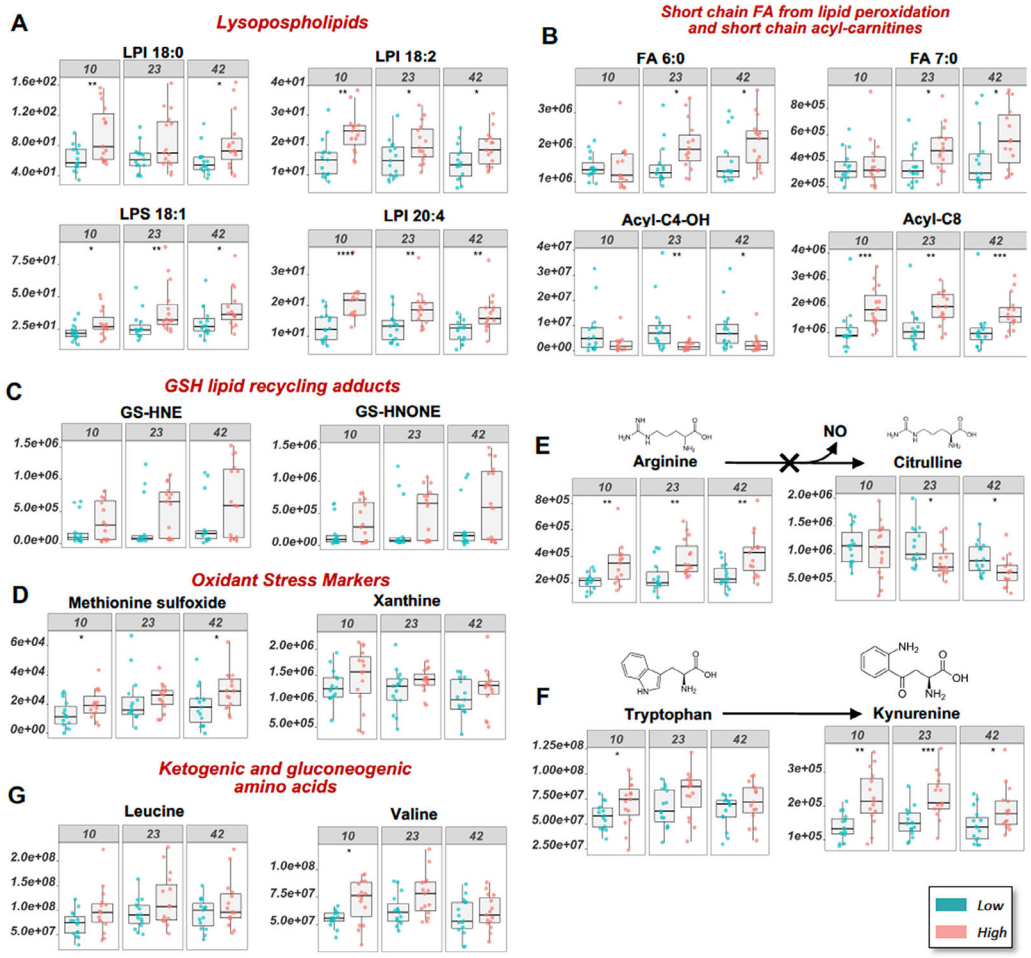

FIGURE 4.

Metabolic signatures of low and high BMI in stored RBCs. Representative metabolites are grouped by classes, including (A) lysophospholipids, (B) short-chain fatty acids and short-chain acyl-carnitines, (C) glutathione adducts, (D) oxidant stress markers, (E) arginine metabolism, (F) tryptophan metabolism, and (G) ketogenic and gluconeogenic amino acids. Metabolites are represented as dot plots with superimposed box and whisker plots, on storage Days 10, 23, and 42. Low- and high-BMI subjects are color-coded according to the legend in the bottom right panel of the figure. Nonparametric Wilcoxon test significance for each time point is indicated (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$)

TABLE 1

Supporting data for Figure 1 summarizing the demographic determinants of blood donors (n = 18) from Vitalant's cohort (Figure 1) at each BMI category

BMI (kg/m^2)	**<25**	**25-29.9**	**≥30**	***P***
Age, years (mean ± SD)				
Figure 1A-C	58.0 ± 11.9	57.1 ± 9.1	52.6 ± 7.0	0.54
Figure 1D	58.7 ± 14.4	57.7 ± 9.9	50.4 ± 7.2	0.43
TRT/controls				
Figure 1A-C	1/3	4/3	4/3	0.53
Figure 1D	1/2	3/3	3/2	0.77
Sex (males/females)				
Figure 1A-C	3/1	7/0	6/1	0.42
Figure 1D	2/1	6/0	4/1	0.36
Ethnicity (white/unavailable)				
Figure 1A-C	4/0	6/1	6/1	0.80
Figure 1D	3/0	5/1	4/1	0.87

Note: P values were obtained by one-way analysis of variance for the continuous variable (age), and χ^2 test was performed for categorical variables (sex, TRT status, and race).

Abbreviations: BMI, body mass index; TRT, testosterone replacement therapy.

TABLE 2

Demographic determinants of blood donors (n = 13 317) who participated in the National Heart, Lung, and Blood Institute red blood cell Omics (RBC-Omics) study between 2013-2015

Race/ethnicity	No. of males	No. of females	Male BMI (mean ± SD)	Female BMI (mean ± SD)	*P* (sex)	Total no.	Total BMI (mean ± SD)
White	4304	4152	28.2 ± 5.0	27.5 ± 6.1	<0.001	8456	27.9 ± 5.6
Hispanic	406	613	28.7 ± 5.2	28.5 ± 6.2	0.56	1019	28.6 ± 5.8
African American	737	857	29.9 ± 6.7	30.2 ± 6.9	0.50	1594	30.1 ± 6.8
Asian	879	733	26.2 ± 3.9	24.9 ± 4.3	<0.001	1612	25.6 ± 4.1
Other	301	335	27.4 ± 4.8	27.6 ± 6.6	0.74	636	27.5 ± 5.8
Total	6627	6690	28.1 ± 5.2	27.7 ± 6.2	<0.001	13 317	27.9 ± 5.7

Note: P (sex) denotes sex differences in BMI.

Abbreviation: BMI, body mass index.

TABLE 3

Distribution of hemolysis measurements, hemoglobin, and ferritin by blood donor BMI and sex

Sex	BMI (kg/m^2)	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	40-44.9	45-49.9	≥50	*P* (ANOVA)[c]
Males	Osmotic hemolysis (%)[a]	27.5 ± 10.5 (N = 28)	28.5 ± 13.1 (N = 1724)	30.8 ± 13.3 (N = 2765)	33.2 ± 14.0 (N = 1248)	32.1 ± 14.2 (N = 413)	32.9 ± 14.1 (N = 129)	31.9 ± 14.5 (N = 32)	31.2 ± 15.9 (N = 21)	<0.001
	Oxidative hemolysis (%)[a]	38.6 ± 9.7 (N = 24)	35.3 ± 10.4 (N = 1387)	37.0 ± 9.8 (N = 2242)	39.5 ± 9.7 (N = 1072)	40.9 ± 10.0 (N = 356)	40.8 ± 7.8 (N = 110)	44.0 ± 10.4 (N = 28)	41.7 ± 8.4 (N = 20)	<0.001
	Hemoglobin (g/dL)[a]	14.8 ± 1.0 (N = 29)	14.7 ± 1.1 (N = 1757)	14.7 ± 1.1 (N = 2809)	14.6 ± 1.1 (N = 1248)	14.7 ± 1.2 (N = 416)	14.5 ± 1.0 (N = 134)	14.4 ± 1.0 (N = 30)	13.8 ± 1.0 (N = 21)	0.002
	Storage hemolysis (%)[b]	0.33 ± 0.16 (N = 28)	0.32 ± 0.18 (N = 1717)	0.34 ± 0.20 (N = 2750)	0.35 ± 0.21 (N = 1240)	0.35 ± 0.22 (N = 411)	0.32 ± 0.19 (N = 129)	0.36 ± 0.12 (N = 32)	0.44 ± 0.33 (N = 21)	<0.001
	Ferritin (ng/mL)[b]	32 ± 32 (N = 29)	42 ± 60 (N = 1796)	43 ± 68 (N = 2877)	45 ± 72 (N = 1278)	52.5 ± 78 (N = 426)	50 ± 84.5 (N = 135)	69.5 ± 94.25 (N = 32)	80 ± 80 (N = 21)	<0.001
Females	Osmotic hemolysis (%)[a]	24.6 ± 11.6 (N= 36)	24.5 ± 12.1 (N = 2530)	26.0 ± 12.1 (N = 1925)	27.8 ± 12.5 (N = 1085)	28.5 ± 13.0 (N = 484)	29.6 ± 12.9 (N = 189)	28.1 ± 13.1 (N = 76)	32.6 ± 11.8 (N = 32)	<0.001
	Oxidative hemolysis (%)[a]	33.1 ± 11.5 (N = 26)	34.1 ± 9.6 (N = 1983)	36.2 ± 9.6 (N = 1581)	37.5 ± 9.3 (N = 893)	39.2 ± 9.9 (N = 419)	40.7 ± 10.2 (N = 160)	39.2 ± 9.7 (N = 65)	41.9 ± 7.7 (N = 28)	<0.001
	Hemoglobin (g/dL)[a]	13.2 ± 0.8 (N = 37)	13.2 ± 0.9 (N = 2614)	13.3 ± 0.9 (N = 1993)	13.3 ± 1.1 (N = 1093)	13.2 ± 0.9 (N = 496)	13.2 ± 1.0 (N = 196)	13.1 ± 0.9 (N = 77)	13.2 ± 1.1 (N = 30)	0.151
	Storage hemolysis (%)[b]	0.29 ± 0.09 (N = 35)	0.29 ± 0.16 (N = 2522)	0.30 ± 0.17 (N = 1922)	0.30 ± 0.17 (N = 1084)	0.30 ± 0.17 (N = 483)	0.32 ± 0.17 (N = 189)	0.32 ± 0.18 (N = 76)	0.31 ± 0.19 (N = 32)	<0.001
	Ferritin (ng/mL)[b]	23 ± 21.8 (N = 38)	21.5 ± 30 (N = 2658)	23 ± 35 (N = 2015)	25 ± 34 (N = 1121)	26 ± 35 (N = 502)	33.5 ± 37 (N = 198)	35 ± 39.5 (N = 79)	31 ± 30.5 (N = 32)	<0.001

Note: Data derived from 13 317 blood donors who participated in the National Heart, Lung, and Blood Institute Red Blood Cell Omics (RBC-Omics) study between 2013 and 2015. The total number of observations is noted by N. Sample numbers may differ among variables, as some data were not available for all subjects in the RBC-Omics database.

Abbreviations: ANOVA, analysis of variance; BMI, body mass index.

[a]Variables with normal distribution reported as mean ± SD.

[b]Variables that are not normally distributed and reported as median ± interquartile.

[c]The *P* values were derived from ANOVA tests across all BMI groups, except for storage hemolysis and ferritin (not normally distributed), in which the *P* values derived from nonparametric Kruskal-Wallis test. We considered *P* values <0.001 as the statistically significant threshold to account for multiple statistical tests.

TABLE 4

Multivariable linear regression modeling of the associations between blood donor BMI and measures of hemolysis

	Storage hemolysis (R^2 = 1.76%)			Osmotic hemolysis (R^2 = 15.4%)			Oxidative hemolysis (R^2 = 10.3%)		
Variables	**β**	***P* value**	**PVE (%)**	**β**	***P* value**	**PVE (%)**	**β**	***P* value**	**PVE (%)**
BMI (kg/m^2)	0.0013	0.0067	11.59	0.35	<0.001	29.1	0.38	<0.001	40.9
Race (White as reference)			7.42			27.1			3.5
African American	0.017	0.056		−14.0	<0.001		−0.99	<0.001	
Asian	0.065	<0.001		−2.68	<0.001		0.67	0.042	
Hispanic	0.052	<0.001		−1.83	<0.001		0.39	0.29	
Other	0.0016	0.90		−3.60	<0.001		0.14	0.76	
Age, y	0.001	<0.001	17.20	−0.01	0.22	0.46	−0.11	<0.001	41.0
Number of donations in past 2 y	0.002	0.041	0.85	−0.20	<0.001	1.42	−0.23	<0.001	5.52
Sex (females as reference)			54.4			35.2			8.01
Males	0.041	<0.001		4.21	<0.001		1.75	<0.001	
Hormone intake (no as reference)			1.96			0.80			0.86
Yes	−0.018	0.067		−1.21	0.002		1.04	0.002	
Ferritin (ng/mL)	0.001	<0.001	6.62	0.01	0.004	5.90	0.004	0.014	0.16

Note: Data derived from 13 317 blood donors who participated in the National Heart, Lung, and Blood Institute Red Blood Cell Omics (RBC-Omics) study between 2013 and 2015. Differences were considered significant at $P < 0.001$ to account for multi-testing after adjustment for donor race/ethnicity, sex, age, donation history in prior two years, sex hormone intake, and donor plasma ferritin.

Abbreviations: β, parameter estimate; PVE, proportion of variance explained (percentage); R^2, the goodness-of-fit measure for each hemolysis measurement.