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The heritability of metabolite concentrations in stored human red blood cells

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Abstract

BACKGROUND—The degeneration of red blood cells (RBCs) during storage is a major issue in transfusion medicine. Family studies in the 1960s established the heritability of the RBC storage lesion based on post-storage ATP concentrations. However, this critical discovery has not been further explored. In a classic twin study we confirmed the heritability of post-storage ATP concentrations and established the heritability of many other RBC metabolites.

STUDY DESIGN AND METHODS—ATP concentrations and metabolomic profiles were analyzed in RBC samples from 18 twin pairs. On samples stored 28 days the heritability of post-storage ATP concentrations were 64% and 53% in CP2D and AS-3 stored RBCs, respectively.

RESULTS—Metabolomic analyses identified 87 metabolites with an estimated heritability of 20% or greater. Thirty-six metabolites were significantly correlated with ATP concentrations ($p < 0.05$) and 16 correlated with borderline significance ($0.05 < p < 0.10$). Of the 52 metabolites that correlated significantly with ATP, 24 demonstrated $\geq 20\%$ heritability. Pathways represented by

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heritable metabolites included glycolysis, membrane remodeling, redox homeostasis and synthetic and degradation pathways.

CONCLUSION—We conclude that many RBC metabolite concentrations are genetically influenced during storage. Future studies of key metabolic pathways and genetic modifiers of RBC storage could lead to major advances in RBC storage and transfusion therapy.

Keywords

red blood cell; storage lesion; genetics; metabolomics; twin study

INTRODUCTION

The safe storage of red blood cells (RBCs) has been a central goal in transfusion therapy for nearly a century.¹ In times of epidemics, natural disasters, war, and unanticipated shortfalls in blood collection, the ability to store and move blood from place to place has been crucial in providing modern medical care. Decades of effort by many investigators have resulted in the development of extended storage solutions and containers that allow storage of RBCs for up to 42 days.¹ Yet, despite marked advances in RBC storage, the variable quality and therapeutic efficacy of stored RBCs remains a major issue in blood banking.^{2,3}

Multiple reports dating back two decades show that the *in vivo* functionality of RBCs deteriorates with time in storage.^{4–9} A provocative study in 2008 concluded that transfusion of RBCs stored longer than 2 weeks was associated with significantly increased post-operative risk and decreased long-term survival in cardiac surgery patients.¹⁰ To rigorously address concerns about RBC quality raised by this study, three major prospective clinical trials are underway to determine possible adverse effects of RBC storage on patient outcomes.³

The deterioration of RBCs during storage, known as the RBC storage lesion, is a complex degenerative process involving alterations of metabolite concentrations, metabolic pathways, biophysical characteristics, and cellular stability resulting in decreased *in vivo* recovery of transfused RBCs.^{2,11–13} *In vivo* recovery studies, which typically measure the recovery after 24 hours of transfused, radiolabeled autologous RBCs in volunteer subjects are required for approval of blood storage devices by the Food and Drug Administration.¹⁴ The biochemical change most predictive of the loss of *in vivo* recovery is the decline of intracellular adenosine triphosphate (ATP).^{15–18} However, it has long been known that the rate at which ATP concentrations and *in vivo* recovery decline during storage is markedly different between individual blood donors.^{2,17,19}

Seminal work in the 1960s by Dern and Wiorkowski,²⁰ and Brewer,²¹ established that post-storage RBC ATP concentrations are largely determined by inheritance. However, the heritability of the RBC storage lesion has not been further elucidated. Based on these historical reports of the heritability of post-storage RBC ATP concentrations, we hypothesized that other RBC metabolite concentrations might also be heritable. To explore this hypothesis we conducted a classic twin study to confirm the heritability of post-storage RBC ATP concentrations and to explore the heritability of other metabolites. Our study

confirmed the heritability of post-storage ATP concentrations and identified numerous additional heritable metabolites. Many of the discovered heritable metabolites correlated significantly with ATP concentrations, suggesting the existence of heritable ATP-linked metabolic pathways. These findings shed light on potentially critical metabolic pathways involved in the RBC storage lesion and provide support for future studies to identify the genes that regulate these metabolomic pathways.

MATERIALS AND METHODS

Twin subject enrollment and sample collection

The study was approved by the Human Subjects office of The University of Iowa Carver College of Medicine. Written informed consent was obtained from all participating subjects. Subjects were qualified for participation by meeting criteria for autologous blood donation according to standard operating procedures of The University of Iowa DeGowin Blood Center. Twin pairs were not required to donate samples at the same time. Standard health history and demographic information was obtained at the time of enrollment and informed consent. Reported height and weight were used to calculate body mass index (BMI). BMI was derived from the formula: $BMI = \text{weight (kg)} / (\text{height(m)})^2$. From these data, the heritability of height, weight, and BMI were calculated as independent assessments of the suitability of our sample population for studies of heritable traits.

Each subject donated one unit of whole blood that was processed according to standard operating procedures into a leukocyte-reduced RBC unit in AS-3 extended storage media (Medsep Corporation, Covina, CA). During processing, integral leukocyte reduction filters were retained for extraction of DNA. The tubing extending from the leukocyte reduction filter to the secondary bag, containing phosphate double dextrose (CP2D) (Medsep Corporation, Covina, CA) anticoagulated RBCs was retained and segmented per standard procedure.

The leukocyte-reduced CP2D segments were stored together with the main RBC unit under standard conditions. Segments were removed for analysis on the first day after donation (day 0), and every 14 days thereafter until day 56. Samples of AS-3 preserved RBC units were prepared from the main unit on each day of sampling. The AS-3 preserved RBCs were sampled by sterile docking of tubing to the RBC unit, back-filling the tubing with RBCs and sectioning into segments. This procedure was performed on the first day after donation (day 0), and every 14 days thereafter until day 56.

Sample preparation ATP for assay

On the day of analysis, tubing segments containing RBCs were transferred to 15 mL conical tubes by cutting the ends of the segments and allowing the RBCs to drain by gravity into tubes. A 250 μL aliquot of RBCs was then transferred to an Eppendorf tube to which was added 375 μL of $1\times$ Dulbecco's Phosphate Buffered Saline (DPBS) and 25 μL of 70% perchloric acid. Samples were mixed and placed on ice for 5 min. Samples were periodically mixed during the 5 min incubation then centrifuged at 14,000 g for 5 min at 4 $^{\circ}\text{C}$. A volume of 417 μL of supernatant was transferred to an Eppendorf tube and 23 μL of 5 M K_2CO_3 was

added to precipitate proteins. The sample was mixed and the CO₂ gas was allowed to escape by opening the cap. The sample was centrifuged at 14,000 *g* for 5 min at 4 °C. The supernatant containing ATP was removed and transferred to new Eppendorf tubes and stored at –80 °C. Samples were prepared and assayed in duplicate.

ATP assay method

The ATP concentration was determined with a kit from DiaSys Diagnostic Systems GmbH (ATP Hexokinase FS cat # 1 6201 99 10 021) based on NADH production. The original protocol was adapted to work with 96-well microplates. Absorbance was measured at 340 nm with a Tecan SPECTRAFluor Plus microplate reader. A standard curve of ATP from 0 to 1 mM was made for ATP quantitation in a 96-well format. Values were normalized to total hemoglobin measured in the same sample prior to protein precipitation (Sysmex XE-2100 Automated Hematology System). Mean values of duplicate ATP determinations were utilized in the analyses.

Zygosity testing

DNA for zygosity testing was obtained from leukocyte reduction filters by rinsing filters with 15 mL DPBS. The rinse volume was centrifuged at 500 *g* for 10 min and the cell pellet was resuspended in 2 mL of DPBS. DNA was extracted from the cell pellet using an AutoGen (Holliston, MA) QuickGene-610L nucleic acid extraction instrument with the Fuji QuickGene DNA Whole Blood Kit (AutoGen).

Genotyping was performed using a previously developed panel of 24 single nucleotide polymorphisms (SNPs). SNP genotyping was performed using TaqMan assays (Applied Biosystems, Foster City, CA) on the EP1 SNP Genotyping System and GT48.48 Dynamic Array Integrated Fluidic Circuits (Fluidigm, San Francisco, CA). Monozygotic (MZ) twins were identified by 90% or greater genotype concordance; all other twin pairs were identified as dizygotic (DZ).

Global metabolomics profile analyses

The untargeted metabolic profiling platform employed for this analysis combined three independent platforms: ultrahigh performance liquid chromatography/tandem mass spectrometry (UHLC/MS/MS²) optimized for basic species, UHLC/MS/MS² optimized for acidic species, and gas chromatography/mass spectrometry (GC/MS).

Sample handling

Aliquots of 100 µL of packed RBCs made in the preparation for the ATP assays were homogenized in 1 mL of nanopure water. Samples were evaporated to near dry and reconstituted into 100 µL of nanopure water. Using an automated liquid handler (Hamilton LabStar, Salt Lake City, UT), protein was precipitated from the samples with methanol that contained four standards to report on extraction efficiency. The resulting supernatant was split into equal aliquots for analysis on the three platforms, as described previously.²² Aliquots, dried under nitrogen and vacuum-desiccated, were subsequently either reconstituted in 50 µL 0.1% formic acid in water (acidic conditions) or in 50 µL 6.5 mM ammonium bicarbonate in water, pH 8 (basic conditions) for the two UHLC/MS/MS²

analyses or derivatized to a final volume of 50 μ L for GC/MS analysis using equal parts bistrimethyl-silyl-trifluoroacetamide and solvent mixture acetonitrile:dichloromethane:cyclohexane (5:4:1) with 5% triethylamine at 60 °C for one hour.

Method controls and blanks

Three types of controls were analyzed in concert with the experimental samples: samples generated from pooled experimental samples served as technical replicates throughout the data set, extracted water samples served as process blanks, and a cocktail of standards spiked into every analyzed sample allowed instrument performance monitoring.

Sample measurement method

For UHLC/MS/MS² analysis, aliquots were separated using a Waters Acquity UPLC (Waters, Millford, MA) and analyzed using an LTQ mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) that consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer. The MS instrument scanned 99–1000 m/z and alternated between MS and MS² scans using dynamic exclusion with approximately 6 scans per second. Derivatized samples for GC/MS were separated on a 5% phenyldimethyl silicone column with helium as the carrier gas and a temperature ramp from 60 °C to 340 °C and then analyzed on a Thermo-Finnigan Trace DSQ MS (Thermo Fisher Scientific, Inc.) operated at unit mass resolving power with electron impact ionization and a 50–750 atomic mass unit scan range. Experimental samples and controls were randomized across a one-day platform run.

Statistics

Metabolite identification and data analysis—Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (m/z), preferred adducts, and in-source fragments as well as associated MS spectra and curated by visual inspection for quality control using software developed at Metabolon.²³ Any missing values were assumed to be below the limits of detection and for statistical analyses and data display purposes, these values were imputed with the compound minimum (minimum value imputation) after normalization to total protein as determined by Bradford assay for each sample.

Heritability Calculations

Heritability estimates were calculated for the change in ATP concentrations during the entire storage period and for ATP and other metabolites on RBC samples stored 28 days. Day 28 of storage was selected as an informative post-storage time point because: 1) the mean age of RBC units transfused at our institution is 23 days, and recent studies report that the majority of RBC units are transfused between 3 and 5 weeks of storage,⁸ 2) a recent metabolomic analysis of stored RBCs indicates that most metabolite concentrations have changed considerably by day 28, but have not reached an asymptotic peak or nadir and should therefore be informative about differences between subjects,¹² and 3) studies by

Dern *et al.* demonstrating the correlation between ATP concentrations and *in vivo* RBC recovery were conducted on RBCs stored 28 days.¹⁷

The one-way model of intraclass correlation coefficient (ICC) was used to determine the similarity of a measure in a twin pair: $ICC = (MS_{\text{between}} - MS_{\text{within}}) / (MS_{\text{between}} + MS_{\text{within}})$, where MS_{between} is the estimate of the mean-square variance between all twin-pairs and MS_{within} is the estimate of the mean-square variance within the sets of pairs in that group.²⁴ The ICC is used to compare the variation within specific pairs to that of the population as a whole, and falls on a scale of -1 to +1. Higher positive values indicate that there is less variation within the pairs of subjects than there would be within randomly paired subjects. Positive values approaching 0, as well as negative ICC values, indicate that the variation within pairs of subjects is similar to the variation expected within random pairs. A strong heritable trait between MZ twins would be expected to have an intraclass correlation coefficient near +1.

The ICC of MZ and DZ pairs for ATP concentrations were calculated using IBM SPSS Statistics version 20. From the ICC values heritability was estimated using the method derived by Newman *et al.*, $h^2 = (ICC_{\text{MZ}} - ICC_{\text{DZ}}) / (1 - ICC_{\text{DZ}})$.²⁵ The mean of the ICC values for all time points (day 0, 14, 28, 42, and 56) for each twin pair was used to calculate the estimated heritability of the change in ATP concentrations during RBC storage.

Metabolite concentrations were log transformed prior to ICC calculation and estimation of heritability (expressed as a %) was performed using R (<http://cran.r-project.org/>). Additionally, correlation analysis was performed using log transformed metabolite concentrations and log transformed ATP concentrations ($\mu\text{mol/g Hb}$) using Array Studio software (Omicsoft, Inc).

RESULTS

Twin subjects and known heritable traits

Among 18 twin pairs, zygosity testing identified 13 MZ and 5 DZ twin pairs. The means of age, weight, and BMI were not significantly different between MZ and DZ twin groups (Table 1). As previously reported, we observed a high degree of estimated heritability for height (96%), weight (97%), and BMI (63%) in this study population.²⁶ The similarity of these results to estimates in previous reports^{27,28} supports the validity of the sample population for determination of heritable traits.

Population trends in RBC ATP concentration during storage

Mean concentrations of ATP in AS-3 stored RBCs rose slightly from day 0 to day 14 of storage, and declined progressively thereafter (Figure 1A). In RBCs stored in CP2D, ATP declined by approximately 75% in an essentially linear manner from day 0 through day 56 of storage (Figure 1B).

Heritability of ATP concentrations during RBC storage

Heritability estimates for ATP concentrations in CP2D-stored RBCs and AS-3-stored RBCs on day 28 of storage were 64% and 53%, respectively (Table 2). The estimate of heritability

of the change in ATP concentration from day 0 to day 56 of storage in CP2D-stored RBCs was 77%, and for AS-3-stored RBCs was 66% (Table 2).

Heritability of metabolite profiles

Metabolomic analyses of RBCs identified 213 known endogenous metabolites for which heritability estimate calculations were performed. Eighty-seven metabolites were identified with an estimated heritability of 20% or greater (Supplemental Table 1). Consistent with heritability of ATP concentrations, ADP concentrations also demonstrated a high degree of heritability (73%). Twenty six metabolites had an estimated heritability of 50% or greater. These results established that many metabolite concentrations are influenced by genetically controlled pathways during RBC storage.

Correlation of metabolites with ATP in RBCs stored 28 days

To explore the hypothesis that post-storage ATP concentrations are genetically co-regulated with other RBC metabolites, we performed correlations between ATP concentrations and the 213 known metabolites profiled in samples stored 28 days. Thirty-six metabolites were significantly correlated with ATP concentrations ($p < 0.05$) and 16 metabolites correlated with borderline significance ($0.05 < p < 0.10$). The vast majority of these metabolites demonstrated an inverse correlation with ATP, with only 5 metabolites, including ADP, demonstrating a positive correlation. Of the 52 metabolites that correlated ($p < 0.10$) with ATP concentrations, 24 also demonstrated $\geq 20\%$ heritability (Table 3). Pathways represented by heritable metabolites that may contribute to the storage lesion include glycolysis, membrane remodeling, redox homeostasis and multiple synthetic and degradation pathways.

DISCUSSION

In the 1960s, pioneering work in the field of blood storage revealed that post-storage RBC ATP concentration is highly heritable.^{20,21,29,30} Because the post-storage ATP concentration is the most informative biomarker of post-transfusion RBC recovery,^{15–18} this seminal work opened the door for studies that can elucidate the molecular and genetic mechanisms of the RBC storage lesion. Our current work confirms and extends the historical observations of Dern and Brewer. In a classic twin study of RBC storage we have observed that ATP concentrations are heritable in RBCs stored for 28 days in both modern (AS-3, heritability = 53 %) and older (CP2D heritability = 64 %) storage solutions. We have also observed that there are many other heritable metabolite concentrations in stored RBCs.

Dern and Wiorkowski observed that pre-storage, as well as post-storage, RBC ATP levels were heritable. They explored the possibility that the heritability of post-storage RBC ATP levels is a direct result of the heritability of pre-storage ATP levels. They concluded, however, that only 36% of the variability in post-storage ATP levels is explained by the heritability of pre-storage ATP levels. They therefore proposed that RBC ATP levels are regulated by at least two genetic mechanisms: one affecting pre-storage ATP levels, and another affecting the levels of ATP under storage conditions.³¹ In the current study, the heritability of ATP levels in RBCs stored in CP2D, which most closely resembles the ACD

formula used by Dern and Wiorkowski, is estimated to be 64 % at day 0 and 64 % at day 28. However, the correlation between pre-storage and post-storage ATP concentrations is not statistically significant (Pearson correlation = 0.13 $p = 0.41$). This observation supports Dern and Wiorkowski's conclusion that pre-storage ATP levels are heritable, but have only a modest influence on post-storage levels. Our data therefore provide further support for the conclusion that there are at least two genetically determined mechanisms that control ATP concentrations during the storage of RBCs.

Presumably, the storage properties of RBCs are not under selective evolutionary pressure. Therefore, the variation in changes in ATP levels during RBC storage may be considered an artificial phenotype resulting from the interaction of the storage environment and the biochemical contents of RBCs at the time cells are collected. It is reasonable to speculate, therefore, that different genes may be more or less influential in different storage conditions and at different times during storage. Our observation of a similar degree of heritability of ATP concentrations in RBCs stored in two different media associated with different profiles of ATP concentrations during storage (Figure 1), suggests that there may be overlap in the sets of genes regulating RBC storage in these two storage conditions. This is corroborated by a significant correlation between ATP concentrations of samples stored in CP2D and AS-3 (Pearson correlation = 0.48, $p = 0.003$). But, the moderate correlation suggests that there may be many differences in the genes influencing storage in these two media. In order to have the broadest clinical impact, future studies of the genetic determinants of RBC storage may do well to focus on genes that influence storage in more than one type of media.

In addition to *in vivo* recovery, a host of *in vitro* changes occur during storage. Many of these changes are related to RBC glucose metabolism. In modern storage solutions, 90% of RBC glucose metabolism occurs anaerobically, generating 2 moles each of lactate, H^+ , and ATP per mole of glucose.^{2,19,32,33} During this process, the pH of the stored cells declines, which directly inhibits the activity of phosphofructokinase, resulting in a gradual slowing of the glycolytic process.² As glycolysis slows, the rate of production of ATP declines and numerous ATP-dependent biological processes are impaired.

RBC ATP-dependent processes include: 1) the release of ATP outside the RBC, which participates in regulation of vascular tone;^{34,35} 2) the generation of 2,3-diphosphoglycerate, which is critical in modulating hemoglobin oxygen affinity;^{2,13,33} 3) the function of the membrane sodium-potassium pump;^{2,13,19,32} 4) the regeneration of glutathione, which is critical for controlling oxidative cellular injury;³⁶ and, 5) the maintenance of membrane composition and membrane-cytoskeletal interactions that preserve cell shape and flexibility.³⁷

To identify metabolic pathways that may be of interest in future studies, we analyzed metabolites from our untargeted metabolic screen that are both heritable and correlated significantly with ATP concentration. It is our hypothesis that such metabolites may be genetically co-regulated with ATP during RBC storage. The identified metabolites were categorized in their respective biochemical pathways. Pathways that emerged from this analysis were: glycolysis, glutathione mediated redox control, lipid metabolism, membrane integrity, and pro-inflammatory eicosanoid synthesis.

Genetic control of glycolysis during RBC storage is reflected by the heritability of glucose-6-phosphate, fructose-1,6-diphosphate (represented as an isobar – when two or more biochemicals have the same retention time, m/z , and GC/MS, or MS/MS spectra such that the individual contributions of the compounds to the peak and the relative concentration cannot be determined, the total concentrations for one or all of the compounds are represented as a isobar), dihydroxyacetone phosphate (DHAP), and pyruvate concentrations (Table 3, Supplemental Table 1). Heritability values greater than 45% for sorbitol, ribulose-5-phosphate and xyulose-5-phosphate (represented as an isobar), and several glycosylation metabolites provide further evidence of genetic control of glucose metabolism. Consistent with the participation of ATP in the generation of fructose-1,6-bisphosphate, concentrations of the isobar containing this key glycolytic intermediate (along with glucose-1,6-diphosphate and myo-inositol(1,4 or 1,3)diphosphate) were positively correlated with ATP concentrations.

Intracellular concentrations of the components of the principal intracellular redox buffer (glutathione and glutathione disulfide) have previously been found to be heritable in fresh RBCs²⁶. In this study, the concentrations of the oxidative stress markers cysteine-glutathione disulfide and oxidized glutathione in day 28 AS-3 stored RBCs were found to be negatively correlated with ATP concentrations, with glutathione disulfide concentrations and the glutathione turnover product 5-oxoproline demonstrating modest heritability (23%–33%) (Table 3, Supplemental Table 1). In addition, concentrations of the oxidized cholesterol species 7- α -hydroxycholesterol and 7- β -hydroxycholesterol and erythronate, which can be produced from the oxidation of glycated proteins, demonstrated greater than 50% heritability, further supporting a genetic component in the maintenance of redox homeostasis in stored RBCs (Supplemental Table 1).

Lysolipids, or single chain glycerophospholipids, are generated by the action of phospholipase A (PLA) on membrane phospholipids. In this study, several lysolipids containing ethanolamine as a head group were found to be significantly correlated with ATP concentrations such that lower concentrations of these species were present in RBC samples with high ATP concentrations (Table 3, Supplemental Table 1). This phenomenon was not observed for lysolipids containing choline as the head group, but was observed for the single inositol containing lysolipid detected, 1-stearoylglycerophosphoinositol. As choline-conjugated phospholipids are most prevalent on the outer leaflet of cellular membranes, while ethanolamine and inositol conjugated phospholipids are enriched in the inner leaflet of membranes, these results may reflect increased degradation and remodeling of intracellular membrane phospholipids in RBCs with depleted ATP stores.

In addition to phospholipid metabolites, the concentrations of several polyunsaturated fatty acids (PUFAs) were found to be both heritable and associated with ATP concentrations in AS-3 day 28 stored RBCs (Table 3, Supplemental Table 1). These fatty acids include the ω -6 fatty acid linoleate (18:2n6) and its derivatives dihomo-linolenate (represented by an isobar of 20:3n3 and 20:3n6) and arachidonate (AA, 20:4n6), as well as the ω -3 PUFA docosapentaenoate (DPA; 22:5n3). As ω -6 PUFAs are precursors to pro-inflammatory eicosanoids, these results support a possible role of inflammatory signaling molecules in the clinical effects associated with transfusion of stored RBCs.⁵

In conclusion, our analysis revealed the heritability of multiple metabolites in stored RBCs. Additionally, a subset of these heritable metabolites is correlated with ATP concentrations in the RBCs, suggesting a potential role for their involvement in the development of the RBC storage lesion. Our work therefore introduces a new genetic facet to the metabolic derangements that occur during RBC storage. With these observations we anticipate to have laid the groundwork for future studies to identify the key genetic and metabolic mechanisms involved in the RBC storage lesion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Changes in RBC ATP concentrations during 56 days of storage
(A) ATP concentrations (expressed in $\mu\text{mol/g}$ of hemoglobin) in RBCs from 36 twin subjects stored in AS-3 media. **(B)** ATP concentrations in RBCs stored in CP2D media. The dashed lines represent median values.

Table 1

Comparison between the mono- and di-zygotic twin populations in this study

Trait	Monozygotic (MZ)	Dizygotic (DZ)	<i>p</i> value *
Female pairs	11	2	
Male pairs	2	2	
Male/female pairs	-	1	
Total pairs	13	5	
Age / Years	25 ± 7 †	26 ± 9	0.7
Weight / kg	68 ± 14	66 ± 8.6	0.6
Height / m	1.68 ± 0.07	1.74 ± 0.06	0.02
BMI	24 ± 4.3	22 ± 2.7	0.11

* Dizygotic versus monozygotic

† Mean ± SEM

Table 2

Estimated heritability of ATP traits in stored RBCs

Trait	Estimated heritability (%) day-28ATP levels [*]	Estimated heritability (%) change in ATP levels [†]
ATP _{CP2D}	64	77
ATP _{AS-3}	53	66

^{*} This represents the heritability of ATP levels in stored RBCs after 28 days.

[†] This represent the heritability of the change in intracellular levels of ATP from day-1 to day-56

Table 3

Heritable metabolites correlated with ATP in RBCs stored 28 days

Metabolites	Correlation with ATP (R value)	Heritability (%)
<u>Amino Acid Metabolism</u>		
4-guanidinobutanoate	-0.41 [*]	27
phenylacetylglutamine	-0.34 [*]	82
proline	-0.33 [*]	23
3-indoxyl sulfate	-0.31 [†]	31
aspartate	-0.31 [†]	41
tryptophan	-0.29 [†]	33
alpha-hydroxyisocaproate	-0.28 [†]	23
<u>ATP Metabolism</u>		
adenosine 5'-diphosphate (ADP)	0.41 [*]	74
<u>Choline and Methionine Metabolism</u>		
betaine	-0.44 [*]	24
<u>Glycolysis</u>		
Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate, myo-inositol 1,4 or 1,3-diphosphate	0.33 [*]	51
<u>Glycosylation</u>		
N-acetylmannosamine	0.41 [*]	54
<u>Membrane Remodeling</u>		
2-oleoylglycerophosphocholine [*]	-0.46 [*]	53
1-stearoylglycerophosphoethanolamine	-0.43 [*]	23
arachidonate (20:4n6)	-0.33 [†]	22
<u>Membrane Remodeling/Redox Homeostasis</u>		
1-palmitoylplasmeneylethanolamine [*]	-0.43 [*]	37
<u>Methylation/Polyamine Metabolism</u>		
5-methylthioadenosine (MTA)	-0.29 [†]	50
<u>Polyunsaturated Fatty Acid Metabolism</u>		
linoleate (18:2n6)	-0.36 [*]	26
dihomo-linolenate (20:3n3 or n6)	-0.37 [*]	45
docosapentaenoate (n3 DPA; 22:5n3)	-0.51 [*]	28
<u>Protein Degradation</u>		
3-methylhistidine	0.33 [*]	51
<u>Purine Metabolism</u>		
allantoin	0.36 [*]	46
adenine	-0.31 [†]	31
uridine	-0.30 [†]	40
<u>Redox Homeostasis</u>		

Metabolites	Correlation with ATP (R value)	Heritability (%)
Glutathione disulfide (GSSG)	-0.46 [*]	33

^{*} indicates that the significance of the R value was 0.05

[†] indicates that the significance was 0.10 and 0.05