Optimization of blood component preparation

Processing and donor influence

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CHAPTER 9

Donor age is associated with platelet storage properties: *in vitro* quality of platelets from young donors and aged donors with and without Type 2 Diabetes

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ABSTRACT

Background and Objectives
Previously it has been shown that platelet (PLT) storage performance is consistent by donor. Differences involved metabolic activity, which might be caused by mitochondrial (dys)function, associated with age and age-related diseases like Type 2 diabetes (T2D). We aimed to test PLTs from young donors in comparison with PLTs from older donors with or without diagnosis for T2D.

Materials and methods
Fifteen whole blood donors <30 year were selected, and single-donor platelet concentrates (sPC) were prepared from buffy coats (BC) and plasma. Also, 2x11 sPC were prepared from matched donors >45 years with and without T2D. The sPC were stored for 8 days and analyzed at regular intervals for in vitro quality.

Results
Donors were 24±3, 60±7 (without T2D) and 59±8 (with T2D) years old. All sPC groups had comparable volume and PLT content. On Day 8, sPC from young donors showed higher pH\textsubscript{37°C} than sPC from older donors (6.84±0.15 vs 6.40±0.48, p<0.01), due to lower lactate production. Also CD62P expression (22.9±7.4 vs 48.8±24.0 %, p<0.01) and HSR reflected better in vitro quality. PLT storage properties of sPC obtained from T2D donors (pH=6.51±0.35), were not different from sPC of matched donors (pH=6.40±0.48). No differences in mitochondrial membrane potential were detected between the groups.

Conclusion
PLTs from young donors exhibited the best storage conditions. On average, PLTs from older donors showed poorer in vitro quality but, considering the sub-optimal storage conditions, the implications for the daily blood bank routine is probably small. No association with T2D was found.
INTRODUCTION

The deterioration of platelet (PLT) quality during storage in plastic containers is well-known as the PLT storage lesion (PSL). In addition to gentle processing steps, the PSL is kept as low as possible by keeping the PLT count and number between certain limits, and by technical measures like gas-permeable containers, continuous shaking and temperature control between 20-24°C [1].

Previously we have shown that, with regard to the PSL, also a donor-related effect can be distinguished. Donors can be classified as having PLTs with good, moderate or poor storage properties [2] and in a pilot study we demonstrated that PLT storage performance was consistent by donor [3]. This was supported by an analysis of quality control data of pH measurements [4]. A main difference between ‘good’ and ‘poor’ storage properties involved glycolytic activity, resulting in a faster decline of pH during storage in ‘poor’ PLT concentrates (PCs). On the cellular level, this may be caused by a different functionality of the PLT mitochondria. Mitochondrial defects have been associated with age and age-related diseases like neurodegenerative (Parkinson’s, Alzheimer’s) diseases, migraine headaches, metabolic syndrome and Type 2 diabetes (T2D) [5]. D’Aurelio et al [6] compared mitochondrial energy state and glycolytic activity of platelets obtained from young and aged individuals. Lactate production was significantly higher in aged subjects, and when the mitochondrial respiratory chain was inhibited, an increase in lactate production was observed, but less pronounced in platelets from the aged individuals. Both observations suggest a relative lower contribution of mitochondria from aged subjects to ATP production. On the donor level there are also indications that donors with ‘poor’-storing PCs, are more likely to have health issues pointing towards Metabolic Syndrome (MetSyn) and T2D [3]. MetSyn is a strong predictor for T2D [7] and is characterized by reduced high density lipoprotein cholesterol (HDL-C), elevated waist circumference, triglyceride levels, blood pressure, fasting glucose and/or drug treatment for one of these health issues. It is also known that PLTs of T2D patients are hyperactivated [8] and have a substantially different proteomic profile, including different expressions of many enzymes involved in carbohydrate metabolism [9].

In the Netherlands, donors who are diagnosed for T2D are allowed to donate blood as long as they use oral medication like metformin, but if people have to inject insulin, they are permanently deferred. The self-reported incidence of T2D by Dutch blood donors of 0.9% is rather low [10], but the chance that the real incidence of T2D will be much higher is realistic, as a later prospective study revealed that the incidence of MetSyn among donors ≥ 45 years of...
age was 23% [11]. In accordance, at the age of 45 years, the Dutch population has a remaining lifetime risk for pre-diabetes of 49% and for T2D of 31% [12]. These more recent data all suggest that the incidence of T2D among our blood donor population is much higher than self-reported by the donors.

We hypothesized that young donors would have PLTs with better storage performance than older donors and we aimed therefore to characterize storage properties of PLTs from whole blood donors below the age of 30 and above 45 years. Because T2D is a chronic disease associated with age, we also wanted to know the effect of T2D on storage performance. To test the hypothesis that donors with T2D would have ‘poor’ storing PLTs, also older donors diagnosed for T2D but not on insulin, were selected and characterized for their PLT storage properties.

**METHODS**

**Preparation of buffy coat-derived single-donor PC**

Whole blood was collected from 15 donors below the age of 30 years, selected for the young group. After overnight hold, the whole blood was separated applying the buffy coat (BC)-method, and BC and plasma were used to make single-donor PCs (sPCs). The sPCs were prepared from the BCs and 60 mL of autologous plasma, as described earlier [13]. To prevent loss of volume and platelets, and based on our experience that contamination with leukocytes is limited and below 10^7 [14], the sPCs were not leukoreduced. To prevent large differences in PLT counts and numbers between the sPC groups, and not to exceed the storage capacity of the container, PLT counts and numbers were adjusted if necessary, to ≤1.1x10^9/mL and ≤80x10^9 respectively. Separately, a group of n = 11 sPCs was prepared and stored in the same way from whole blood of donors older than 45 years, with the concomitant preparation of an equivalent number of sPCs from whole blood of T2D donors. The latter were previously diagnosed with T2D, which was self-reported to the blood bank physician. Selection was based on the medical records in the Blood Bank Information System and performed by checking day to day if T2D donors had donated. Donors with T2D were matched in the same collection session with donors of the same age (<5 year difference) and gender. All sPCs were stored until Day 8 at 22±2°C in a 600 mL PVC-DEHP container (P4201, Fresenius Kabi, Bad Homburg, Germany) on a flatbed shaker and sampled on Day 1, 4 or 5 and 8. The older groups and the young group were investigated during the autumn of 2016 and 2017 respectively, and consent from the donor was part of the donor questionnaire.
In vitro analysis of sPCs

The sPCs were weighed and sampled using a swan-lock adapter (Codan ref.16.4200, Lensahn, Germany) and syringe for determining the in vitro quality. At first, the swirling effect was judged (0 = no swirl, 3 = excellent swirl) and pH, blood gases, glucose and lactate was determined with a blood gas analyzer at 37°C (Radiometer ABL90, Radiometer, Copenhagen, Denmark). Platelet count, mean PLT volume (MPV) and leukocyte count were determined with a blood cell analyzer ( Sysmex XT2000i, Kobe, Japan).

Flow cytometric assays were used to determine expression of the activation marker CD62P with fluorescently labeled anti-CD62P [2], binding of fluorescently labeled Annexin A5 as a marker for phosphatidylinerine (PS) exposure [15] and the mitochondrial membrane potential (MMP) with the dye JC-1 [3,16]. The MMP was expressed by the ratio of mean fluorescence intensity of the FL2 and FL1 detectors. A FACS Calibur (BD Biosciences, San Jose, CA) was used and assays were performed as earlier described. In brief, FITC-labeled anti-CD62P (BD Biosciences) was used, and FITC-labeled IgG1 (Beckman Coulter, Fullerton, CA) was used as negative control, in samples diluted with FACSFlow (BD Biosciences). After incubation for 20 minutes at room temperature, PLTs were fixed with formaldehyde (0.5%) and measured within 30 minutes. Annexin A5-FITC (VPS Diagnostics, Hoeven, The Netherlands) was used for labeling PLTs, diluted with HEPES medium supplemented with Ca²⁺ for the test; platelets induced with A23187 served as positive control. Platelets in a medium supplemented with the calcium chelator ethylene glycol tetraacetic acid served as negative control. After incubation for 30 minutes at room temperature, PLTs were further diluted with the corresponding medium and measured within 30 minutes. Samples for MMP measurement were diluted in PAS-E, incubated with JC-1 dye (final concentration 0.5 µmol/L) at 37°C for 20 min in a temperature controlled mixer, and after further dilution in ice-cold PAS-E measured within 15 minutes.

The hypotonic shock response (HSR) was measured in triplicate using a Chronolog aggregometer (CH490, Havertown, PA). When pH in the sPC was <6.5 on Day 8, a sample was cultured (BacT/Alert, BioMerieux, Marcy-l’Etoile, France) to verify that units were not contaminated with bacteria.

Determination of malate and nucleotides

Afterwards, supernatants of sPCs from young donors and 12 selected good or poor storing sPCs from older donors, 2x3 from each group, were analyzed for the extracellular malate concentrations as follows: supernatant of sPCs was diluted with PBS (ratio 2:3) and
deproteinized with 70% perchloric acid (4% v/v). Malate in neutralized extracts was converted by malate dehydrogenase, in a buffer containing hydrazine sulfate and glycine and excess nicotinamide adenine dinucleotide phosphate (NAD+), in oxaloacetate-hydrazine and NADH. Changes in fluorescence at 450 nm were detected after excitation at 340 nm (Infinite 200 Pro, Tecan, Männedorf, Switzerland). In addition, extracts of the good or poor storing sPCs were analysed with high performance liquid chromatography (HPLC) to assess the level of nucleotides, as described earlier in detail [3].

**Analysis of plasma and red cells**

Total cholesterol, HDL-C and triglycerides were measured on a Roche Cobas 8000 c502 or c702 (Roche Diagnostics, Basel, Switzerland) from CPD-plasma samples of Day 1 (frozen and stored at -30°C), using standard colorimetric methods. LDL cholesterol (LDL-C) was calculated using the formula of Friedewald: \[\text{[LDL-C]} = \text{[Total-C]} - \text{[HDL-C]} - \left(\text{[Triglycerides/2.2]}\right)\], with all concentrations in mmol/L [17].

The diabetic marker HbA1c was determined from the red cells with a Tosoh G8 HPLC analyser (Sysmex). Tubes were stored at +4°C and analyzed within 7 days, as validated.

**Statistical analyses**

The distribution of data sets were checked, and when they were not normally distributed, data are given as median and range, otherwise, data are given as mean ± standard deviation. Differences between the three groups were analyzed with a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison post-test. A Kruskal-Wallis test followed by a Dunn’s multiple comparisons test was used when data were not normally distributed (Instat, Version 3.06, GraphPad, San Diego, CA). The comparison of PLT storage data of, ‘good’ versus ‘poor’ sPCs from older donors, was performed using an unpaired one-sided t-test (Microsoft Excel version 2013). When data were suspected of being an outlier, Grubb’s test was used for confirmation.
Platelets from young and diabetic donors

RESULTS

Donor characteristics
Donors <30 year had lower blood pressure and total cholesterol levels than donors >45 year (Table 1). One of the young donors had a very high triglyceride level (3.4 mmol/L) which was determined as an outlier. As intended, diabetic donors were of comparable age to the older control group. Donors with T2D had a higher body mass index (BMI) and higher diastolic blood pressure before donation than their age-matched controls, but cholesterol (total, HDL-C, LDL-C) and triglyceride levels were not significantly different between these groups.

Table 1: Donor demographics and results from plasma and red cell measurements (mean±SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young &lt;30 y n = 15</th>
<th>Older &gt;45 y n = 11</th>
<th>Older Diabetes &gt;45 y n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>24 ± 3</td>
<td>60 ± 7c</td>
<td>59 ± 8c</td>
</tr>
<tr>
<td>Male / Female</td>
<td>10 / 5</td>
<td>8 / 3</td>
<td>8 / 3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4 ± 3.0</td>
<td>25.6 ± 3.5</td>
<td>30.5 ± 4.8</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>128 ± 13</td>
<td>148 ± 17h</td>
<td>154 ± 18c</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75 ± 7</td>
<td>84 ± 8f</td>
<td>93 ± 8f</td>
</tr>
<tr>
<td>Cholesterol and lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.4 ± 0.6</td>
<td>4.2 ± 0.7a</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>1.8 ± 0.5</td>
<td>2.3 ± 0.6</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides, mmol/Lf</td>
<td>0.7 , 0.6-0.9</td>
<td>0.9 , 0.8-1.1</td>
<td>1.2 , 1.1-2.0f</td>
</tr>
<tr>
<td>Red cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>34.1 ± 3.3</td>
<td>38.6 ± 4.9</td>
<td>52.8 ± 7.9</td>
</tr>
</tbody>
</table>

*p<0.05; b p<0.01; c p<0.001 versus Young; d p<0.05; e p<0.001 versus Older;
f median, interquartile range

All young donors had HbA1c <42 mmol/mol and all diabetic donors, diagnosed between 2007 and 2016, had HbA1c >42 mmol/mol (Table 1, Figure 1). Also 2/12 older control donors had HbA1c >42 mmol/mol, above the normal range (20-42 mmol/mol). Seven T2D donors used metformin, and of these, 2 donors also used gliclazide; 3 donors did not use medication (anymore) and from one donor, use of medication was not known.
Figure 1: HbA1c levels of young donors (△) were all within the normal range (20-42 mmol/L, horizontal lines). HbA1c of older donors with Type 2 diabetes (◊) were significantly higher than HbA1c levels of older donors (X) and all above the normal range.

PLTs of young donors showed the best storage performance

The sPCs in all groups had similar volume and PLT content (Table 2) and all sPCs except one in the older group, had normal PLT concentration, >0.8x10⁹/mL and <1.2x10⁹/mL. The leukocyte numbers were low (3.6x10⁶, 0.7x10⁶-21.5x10⁶ per unit) and comparable in all three groups.

Mean pH in sPCs from young donors on Day 8 was significantly higher than in both older groups (Table 2). Of note, the variation in PCs was much higher in both older groups than in the young group (Figure 2). The young group contained only one sPC with low pH (6.37) from a donor with a very low level of HbA1c (26.4 mmol/mol). The older group contained 5 and the diabetic group 3 sPCs with a pH below 6.3, the lower limit suggested by the Council of Europe [18] and recalculated for pH measurements at 37°C. Also other variables in the young group reflected better in vitro quality than in the older groups. At the end of storage, swirling score was 2-3 in the young group but 0-3 in the older groups (p<0.05, ANOVA). Glucose consumption and lactate production were lower and the PLTs showed significantly less activation (CD62P expression) and a better HSR. Differences in MMP were not observed throughout storage, but units with low pH in all groups ended also with low PLT MMP on Day 8 (see below). Bacterial screening of sPCs with pH<6.5 after storage showed no growth in all units.
Table 2: In vitro quality of single-donor platelet concentrates prepared from whole blood, stored at 22±2°C (mean±SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day*</th>
<th>Young &lt;30 y n = 15</th>
<th>Older &gt;45 y n = 11</th>
<th>Older Diabetes &gt;45 y n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, mL</td>
<td>1</td>
<td>71 ± 3</td>
<td>72 ± 2</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>PLT, x10⁹</td>
<td>1</td>
<td>73 ± 8</td>
<td>74 ± 11</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>PLT, x10⁹</td>
<td>8</td>
<td>60 ± 8</td>
<td>60 ± 4</td>
<td>55 ± 7</td>
</tr>
<tr>
<td>MPV, fL</td>
<td>1</td>
<td>9.0 ± 0.8</td>
<td>9.3 ± 0.8</td>
<td>9.6 ± 0.8</td>
</tr>
<tr>
<td>MPV, fL</td>
<td>8</td>
<td>10.1 ± 1.1</td>
<td>11.4 ± 2.1</td>
<td>11.5 ± 1.3</td>
</tr>
<tr>
<td>pH (37°C)</td>
<td>1</td>
<td>7.04 ± 0.03</td>
<td>7.08 ± 0.04b</td>
<td>7.07 ± 0.05</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>8</td>
<td>6.84 ± 0.15</td>
<td>6.40 ± 0.48e</td>
<td>6.51 ± 0.35b</td>
</tr>
<tr>
<td>Glucose consumption, mmol/day/10 PLT</td>
<td>1-8</td>
<td>0.08 ± 0.02</td>
<td>0.15 ± 0.07e</td>
<td>0.14 ± 0.04e</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>1</td>
<td>5.4 ± 0.9</td>
<td>5.3 ± 1.3</td>
<td>5.8 ± 1.2</td>
</tr>
<tr>
<td>Lactate production, mmol/day/10 PLT</td>
<td>8</td>
<td>15.3 ± 2.8</td>
<td>23.4 ± 9.3e</td>
<td>21.9 ± 6.1b</td>
</tr>
<tr>
<td>pO₂, mm Hg</td>
<td>1</td>
<td>143 ± 7</td>
<td>141 ± 12</td>
<td>142 ± 11</td>
</tr>
<tr>
<td>pCO₂, mm Hg</td>
<td>8</td>
<td>27 ± 18</td>
<td>32 ± 22</td>
<td>27 ± 16</td>
</tr>
<tr>
<td>JC-1, ratio FL2/FL1</td>
<td>1</td>
<td>10.5 ± 1.8</td>
<td>12.2 ± 2.7</td>
<td>11.5 ± 2.2</td>
</tr>
<tr>
<td>CD62P expression, %</td>
<td>8</td>
<td>4.7 ± 1.8</td>
<td>4.4 ± 2.9</td>
<td>6.9 ± 5.0</td>
</tr>
<tr>
<td>Annexin A5 binding, %</td>
<td>1</td>
<td>16.6 ± 10.0</td>
<td>14.8 ± 9.5</td>
<td>16.4 ± 8.3</td>
</tr>
<tr>
<td>HSR, %</td>
<td>8</td>
<td>22.9 ± 7.4</td>
<td>48.8 ± 24.0e</td>
<td>36.8 ± 17.9</td>
</tr>
<tr>
<td>Median, interquartile range</td>
<td>1</td>
<td>10.5 ± 1.8</td>
<td>12.2 ± 2.7</td>
<td>11.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4.7 ± 1.8</td>
<td>4.4 ± 2.9</td>
<td>6.9 ± 5.0</td>
</tr>
</tbody>
</table>

* Day 8: sPC in Older group with PLT<0.8x10⁹/mL was excluded

**p<0.05; ***p<0.01; ****p<0.001 versus Young; ****p<0.05 versus Older

Figure 2: pH on Day 8 of single-donor PCs obtained from young donors (Δ), older donors (x) and older donors with diabetes (○). Units from donors with HbA1c >42 mmol/mol are also marked with a square (□). Cut-off values for age are marked with dashed lines and the lower limit for good in vitro quality as a dotted line.
Comparing the diabetic group with the age matched group on Day 1, the glucose concentration was slightly higher in the diabetic group. At the end of storage, mean pH in the diabetic and age matched group was comparable, and just like the other \textit{in vitro} variables, not significantly different between the groups. Overall glycolytic activity, as reflected by glucose consumption and lactate production was comparable in both groups.

\textbf{Good versus poor storing PLTs of older donors}

Both older groups, with and without T2D, showed a broad range of \textit{in vitro} quality, and both groups contained units with good, moderate and poor storage performance. Based on this classification in earlier studies [2,3], and in search for biological and biochemical factors, which could explain the difference in platelet storage performance, from both groups 3 ‘good’ (pH_{day8}>6.6) and 3 ‘poor’ (pH_{day8}<6.3) storing sPCs were selected, and these 2x6 sPCs were analyzed post-hoc in more detail. The ‘good’ and ‘poor’ storing groups had similar volume and composition of the sPCs and corresponding donors had similar demographic characteristics and HbA1c levels (data not shown). All three ‘poor’ T2D donors and one ‘good’ T2D donor used metformin, while two ‘good’ T2D donors used no medication. However, triglyceride levels were significantly higher in ‘poor’ sPCs (2.2±0.7 vs 1.1±0.2 mmol/L, p<0.01).

As shown in Figure 3A, pH values in ‘good’ and ‘poor’ storing PLTs diverged already on Day 4, due to an, on average, 2.5x higher lactate production in ‘poor’ sPCs (Fig 3B). This difference on Day 4 was not accompanied by a significant change in MMP as measured with the fluorescent probe JC-1. However, ‘poor’ PLTs did not maintain their MMP throughout storage, in contrast to the ‘good’ storing PLTs (Fig 3C). In addition, extracellular malate concentrations in ‘good’ sPCs were rather stable, but in ‘poor’ sPCs an increase was observed during storage (Fig 3D), resulting in higher average concentrations on Day 8 (17.9±4.1 vs 11.6±4.1 µmol/L, p<0.05). For comparison, free malate in sPCs from young donors, which were scored as good storing PLTs, was 4.1±1.4 µmol/L.

ATP levels were comparable on Day 1, and were 52.7±7.5 for ‘poor’ versus 51.0±7.0 µmol/10^{11} PLT for ‘good’ donors, but lower in ‘poor’ PLTs on Day 8: 32.3±5.9 vs 43.0±2.4 µmol/10^{11} PLT, p<0.01. Data of ADP and AMP levels are not shown, but were not different between the groups at any time point. Pronounced differences in CD62P expression (65.8±18.5 vs 27.9±6.9%, p<0.001), and Annexin A5 binding (57.7±15.7 vs 20.1±9.1%, p<0.001) reflected the differences in PSL between ‘poor’ and ‘good’ storing PLTs.
DISCUSSION

In this study, we observed that storage properties of PLTs obtained from young donors <30 year were better than from older donors >45 year, who have on average higher BMI, blood pressure, cholesterol and triglyceride levels and HbA1c. In contrast to the young donor group, the older donor groups with or without T2D, showed a broad range of *in vitro* quality after the 8-day storage period. Storage performance of sPCs from whole blood of donors with non-insulin dependent T2D was not different from sPCs of age and sex matched controls, which means that our second hypothesis, namely that donors with T2D would have ‘poor’ storing PLTs, has to be rejected.

Remarkably, our observations are contradictory to the conclusion drawn from quality control data of apheresis PLTs that a low pH was more often measured in PCs obtained from young donors [4]. However, this was only a small effect in a retrospective study, which may be due to confounding factors like a higher total PLT count for younger donors or a
higher proportion of healthy donors in the elder PLT apheresis donor group. On the other hand, our data showed a broad range in the outcome of the older donors, suggesting that the association of age with platelet storage properties is individually determined.

The underlying cellular mechanism which determines platelet storage performance is most likely the lower glycolysis rates in PLTs from young donors, in agreement with our previous study [3]. The higher lactate production and also the great variability in sPCs from older donors confirm the observations of D’Aurelio et al [6] in washed PLTs. As their experiments demonstrated, there are indications that this is caused by partial mitochondrial dysfunction, which is also the case in our study, because all ‘poor’ PLTs ended with a low MMP after 8 days of storage. In addition, secretion of malate was linked to mitochondrial metabolic activity and the disability to consume it [19]. They observed, beside increasing lactate, also increasing extracellular malate concentrations, and both metabolites showed high variability. In our case, the extracellular malate concentrations were significantly higher in ‘poor’ PLTs, which is indicative for a relatively low contribution of the tricarboxylic acid (TCA) cycle to catabolism [19]. This may be associated with the higher triglyceride levels in ‘poor’ donors, which may be caused by a lower rate of β-oxidation taking place in the mitochondria. In addition, the outlier in the young donor group was also characterized by the highest lactate production and lowest MMP on Day 8 in this group. Taken together, these observations are pointing to glycolysis as the preferred pathway for intracellular energy production in ‘poor’ storing donors.

A causal relationship between T2D and ‘poor’ PLT storage properties could not be determined, but besides 3 donors with T2D, also the 2 older control donors with high HbA1c had poor storing PLTs. So, an association of (pre)diabetes with poor platelet storage may exist, but is also individually determined by other factors. The comparison of ‘good’ versus ‘poor’ sPCs, showed that donors with ‘poor’ storing PLTs had higher triglyceride levels, which is pointing towards an impaired triglyceride metabolism as another cause for poor PLT storage performance. Real triglyceride levels in donor plasma were about 20% higher, due to dilution with the CPD anticoagulant. When the corrected levels are compared with non-fasting levels of Dutch subjects [20], it can be concluded that 4/6 ‘poor’ donors had triglyceride levels in the upper-quartile range of >2.5 mmol/L. It is therefore not unlikely that ‘poor’ donors suffered from hypertriglyceridemia. However, more research is needed, and further studies should also investigate red cell quality of ‘good’ vs ‘poor’ donors, because red cell fragility is dependent on triglyceride levels and type of fatty acids incorporated [21].
The lack of difference in PSL between older donors without T2D and with controlled/treated T2D, may be caused by many confounding factors and differences between individuals, like genetics, diet, the use and dose of medication and behavior like sporting and smoking. All of these items may have influence on metabolism and health status of the donor, and on PLT function and metabolism during storage in a bag [22,23]. Another confounder in this study is that the HbA1c level can be reduced up to 11.9% due to blood donation [24], indicating that the real diabetic state of donors diagnosed for T2D or not, was most likely underestimated. According to literature, HbA1c is also reduced by the use of metformin [25].

As mentioned before, MetSyn and T2D are widely spread among the Dutch (donor) population, and the match-controlled comparison revealed roughly 25% ‘poor’ donors. In our country, PLT production is for more than 90% based on pooled BCs, and inclusion of a BC from a ‘poor’ donor might have only a small effect. However, for countries with PLT production mainly based on apheresis, this may be important information in relationship to national epidemics concerning T2D. A study in Texas for instance, showed a prevalence of prediabetes and diabetes of 11% in a large cohort of donors of age 16-19 years [26]. To establish a causal relationship of poor storage of PLT with (pre)diabetes and/or hypertriglyceridemia, further research is warranted. It is recommended to perform prospective studies, including more detailed characterization of donors.

A limitation of this study are the numbers tested, which may be not large enough to prove superiority of platelet storage performance of young donor platelets over those from older donors. This also warrants follow-up studies. Another limitation is the fact that single-BC PCs were stored under sub-optimal conditions in a PVC-DEHP container and not in a pool of 4 or 5 BC. Nevertheless, pre-selection of donors who have low lactate producing PLTs, may help to improve in vitro quality of PCs, and thereby recovery and survival after transfusion, as was demonstrated by Goodrich et al [27] for Mirasol-treated PLTs. In addition, improvement of PLT in vitro quality creates opportunities to extend PC shelf life, to prevent discarding of high-yield PLT units and to improve the quality of pathogen-reduced PCs. In conclusion, PLTs from young donors exhibited the best storage conditions. PLTs from older donors showed poorer in vitro quality, tending to be below average. Considering the sub-optimal storage conditions, the implications for the daily blood bank routine is probably small, since platelets are stored in gas-permeable containers with gentle agitation. However, the clinical implications are not known. We did demonstrate no association with T2D, but our finding could be related to other diseases, and this should be an area of further research.
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REFERENCES

13. Van der Meer PF, de Korte D. Active cooling of whole blood to room temperature improves blood component quality. Transfusion 2011;51:357-362.


