Donor body mass index is an important factor that affects peripheral blood stem cell yield in healthy donors after mobilization with granulocyte-colony stimulating factor

Running head: Body mass index affects stem cell yield

Abstract:

BACKGROUND: Hematopoietic stem cell (HSC) transplantation is increasing rapidly in recent year. Peripheral blood stem cell (PBSC) collection has become the major means for HSC transplantation. However, the response to PBSC mobilization varies and sometimes causes mobilization failure.

STUDY DESIGN AND METHODS: A retrospective study of 69 healthy donors who underwent allogeneic PBSC donation by leukapheresis was performed. All of these donors received 10μg/kg/day granulocyte-colony stimulating factor (G-CSF) for 5 days before PBSC harvest. Donor factors were evaluated and compared between donors with different mobilization responses as indicated by the pre-collection CD34 count (pre-CD34).

RESULTS: Donors with a pre-CD34 over 100/μL had higher body mass index (BMI) than that of donors whose pre-CD34 was 38 to 99/μL or below 38/μL (32.0±1.04 vs. 28.7±0.93 vs. 25.9±1.27 kg/m^2, respectively) ($p < 0.05$). The obese or overweight donors had higher pre-CD34 count on a per-kg of body weight basis than that of normal weight donors. And there was no difference in pre-CD34/kg between females and males when their BMI was similar.

CONCLUSION: Body mass index is an important factor that affects donor's response to mobilization, which may be due to a relatively high dose of G-CSF or unknown intrinsic factors associated with obesity.

Key words: stem cell transplantation, peripheral blood stem cell, mobilization, healthy donors, body mass index

ABBREVIATIONS: CBC = complete blood count; G-CSF = granulocyte-colony-stimulating factor; HSC = hematopoietic stem cell; PBSC = peripheral blood stem cell; pre-CD34 = pre-collection CD34 positive cell count

INTRODUCTION

Hematopoietic stem cell transplantation has been accepted as an important strategy for the treatment of many hematologic diseases,[1] such as acute leukemia, lymphoma, and multiple myeloma, as well as sickle cell disease,[2] and beta thalassemia major. [3,4] It has also been extensively studied for non-hematologic diseases and promising results had been obtained in the treatment of some autoimmune disorders such as systemic lupus erythematosus, [5] CNS tumor[6], renal cell carcinoma and other solid tumors. [7]

Although bone marrow was initially the sole source for hematopoietic stem cells, leukapheresis of peripheral blood stem cells (PBSCs) after mobilization has exceeded bone marrow stem cell collection recently. [8,9] Compared to bone marrow harvests, PBSC harvests have several advantages, including shorter time to engraftment, faster immune reconstitution, [10] potentially better graft-vs-leukemia effects, [11] easier graft manipulation, and decreased risk of tumor contamination. Therefore, autologous transplants now rely almost exclusively on PBSC rather than marrow.

One of the biggest hurdles in PBSC collection is the yield of stem cells, which highly relies on the response to marrow mobilization.[12,13] In the last decade, great efforts had been made on selecting mobilizing reagents and regimes. [12,14] Granulocyte-colony-stimulating factor (G-CSF) has been the most commonly used reagent, although new reagents are being extensively studied because of frequent mobilization failure with G-CSF especially in autologous donors. Moreover, it has been shown that there is great inter-individual variation in hematopoietic stem cell mobilization even among healthy individuals. [15] However, there are still some controversies in term of the effects of donor age, gender, body mass index, etc. [16-18]

In the present study, we aimed at defining donor factors that contribute to a good response to G-CSF mobilization which can then be used to choose appropriate donors for PBSC donation. This information will help to investigate the intrinsic factors that may be modified to improve a donor's response to mobilization.

MATERIALS AND METHODS

Donors

A retrospective chart review (apheresis flow sheets, clinical laboratory records, and stem cell transplant consults) was performed on a total of 69 healthy volunteer allogeneic donors who underwent PBSC donation between November 2009 and December 2011. The donor characteristics are summarized in Table 1.

Mobilization and Collection of PBSC

All donors were administered human recombinant G-CSF (filgrastim) 10 μg/kg/day subcutaneously for 5 days to mobilize PBSC. On day 5, PBSC harvest was performed on the COBE spectra (Caridian BCT, Lakewood, CO). A minimum of 12 L of whole blood was processed with anticoagulant Citrate Dextrose Solution (ACD-A) in approximately 4 hours. The decision whether and how much to extend the procedure was made between the medical director and the donor based on the pre-collection CD34 count. No procedure exceeded a total of 24 L of whole blood processing no matter what the CD34 count was.

Clinical Laboratory Test

A baseline complete blood count (CBC) with differential was obtained as a part of the donor workup performed around 3 weeks before PBSC collection. Blood samples were drawn immediately prior to

apheresis and sent to the clinical hematology laboratory for stat CBC with differential and the flow cytometry laboratory for CD34 positive cell count. Post-apheresis blood samples and collected PBSC products were tested in the same manner.

Statistical Analyses

Data were analyzed with the SPSS 17.0 software package (IBM, Chicago, IL). Where not indicated otherwise, variables were recorded as mean values and standard deviation, and a *t* test and one-way analysis of variance were applied. Correlation of pre-collection CD34 count and final CD34 cell yield was analyzed by the Pearson correlation. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Correlation between pre-CD34 count and CD34 positive cell collected

It has been shown that the CD34 positive cell count as measured immediately prior to leukapheresis (pre-CD34) has a strong correlation with the amount of CD34 positive cells collected at the end of leukapheresis.[19,20] In order to determine the correlation strength between pre-CD34 and CD34 yield after leukapheresis in our donor population, we studied 42 donors who underwent 12 liters of blood processed. (Figure 1) The pre-CD34 strongly correlated with CD34 cells collected after 12 L of blood processing ($p < 0.001$) with a correlation coefficient (r) of 0.86, which is similar to previous reports. [19,21] These results indicated that pre-CD34 count is the best predictor of final CD34 yield as well as a reliable indicator of bone marrow mobilization efficiency. Thus, pre-CD34 was employed in this study to investigate the donor factors that may affect bone marrow mobilization with G-CSF.

Overweight and obese donors have higher Pre-collection CD34/kg and final CD34 yield

To investigate donor factors that affect bone marrow mobilization, the donors were divided into 3 groups based on their pre-CD34 count; poor responders had a pre-CD34 below 38/μL; good responders had pre-CD34 above 38/μL but below 100/μL; and excellent responders had pre-CD34 of 100/μL or above. First, we found that a higher body mass index (BMI) was associated with a higher pre-CD34. Excellent responders had higher BMI (32.0 ± 1.04 kg/m^2), compared to that of good responders (28.7 ± 0.93 kg/m^2) group or poor responders (25.9 ± 1.27 kg/m^2) ($p < 0.05$). (Figure 2A) The differences in BMI between these 3 responder groups were more prominent in female donors. (Figure 2B)

Comparison of pre-CD34 count on a per-kg weight basis among responder groups

Next we compared the pre-CD34 count on a per-kg body weight basis among these donors. The total circulating CD34 positive cells prior to collection was determined by pre-CD34 and donor's total blood volume as calculated with Nadler's formula. Then the pre-CD34 per kg of donor body weight was calculated and compared between the 3 donor groups. (Figure 3A) The excellent responders had the highest pre-CD34/kg (8.87 ± 0.89; x 10^6/kg), which is significantly higher than that of good responders (3.63 ± 0.17; x 10^6/kg) ($p<0.001$) and poor responders (1.62 ± 0.11; x 10^6/kg) ($p<0.001$). However, the pre-CD34 count per Kg of donor weight was similar between female and male donors in the poor and good responder groups. (Figure 3B) The statistical analysis was not performed between female and male in the excellent group because there were only 2 female donors in this group.

Total blood volume processed and final CD34 yield in different responder groups

In the majority of PBSC transplantations, the number of PBSC requested are $5x10^6$ cells per kg of recipient weight. In order to achieve the collection goal, the procedures had often to be extended because of the difference between recipient's and donor's body weight and donor's response to mobilization. Actually, the pre-CD34 count has been widely used in the evaluation of donor's response

to mobilization. In our facility and many other institutions, pre-CD34 count was also used to determine the blood volume to be processed to achieve the collection goal. In the excellent responder group, all of the collections were completed with a standard 12 L blood volume processing. Whereas the procedure was often extended in the other two groups in order to achieve collection goal. (Figure 4A) The mean blood volume processed was 18.6 (± 1.15) L in poor responder group and 13.8 (± 0.59) L in good responder group. In contrast, the actual harvested CD34 cells were still less in poor and good responder groups even with extended collection than that of excellent responders (333 ± 36 vs. 536 ± 26 vs. 826 ± 85; x 10^6, respectively). ($p < 0.05$)(Figure 4B)

Obese or overweight donors had higher pre-CD34/kg and final CD34 yields

The above results indicated that donors with better response to mobilization tend to be heavier and there was no significant difference in donor's age or gender in each responder group. Next, to address the question whether heavier donors have a better response to marrow mobilization and subsequently a better PBSC yield, we divided our donors into 3 groups based on their BMI. In donors with normal weight as defined by a BMI between 18.5 to 24.9 kg/m^2, the pre-CD34 count per kg of body weight was lower than that of overweight (BMI between 25 to 29.9 kg/m^2) and obese (BMI over 30 kg/m^2) donors (2.84 ± 0.42 vs. 4.40 ± 0.68 vs. 5.62 ± 0.73; x 10^6/kg, respectively)(Figure 5A) Because of the lower pre-CD34 in donors with lower BMI, the collection procedure was often extended. The mean of blood volume processed in low BMI donors was 17.0 (± 1.12) L vs. 14.3 (± 0.88) L in overweight and 13.1 (± 0.57) L in obese donors. (Figure 5B) The final PBSC yield depends mainly on the pre-collection circulating CD34 cells, and in a much less extent, on the blood volume processing. It is not surprised to obtain the highest CD34 cells from obese donors (683 ± 58; x 10^6), when compared to that from normal weight donors (419 ± 43; x 10^6) and overweight donors (503± 44; x 10^6). (Figure 5C) Interestingly, there was no difference in pre-CD34/kg between female and male donors when their BMI was similar. (Figure 5D)

DISCUSSION

PBSC collection by leukapheresis has become the primary method in harvesting hematopoietic stem cell for transplantation. Compared to the bone marrow stem cell donation, a successful PBSC harvest depends on multiple factors including mobilization protocols, harvest procedures, and previous chemotherapy history or cancer status in autologous donation. Moreover, previous studies have shown that the stem cell dose is critical in influencing the outcome of hematopoietic stem cell transplant.[22] The infusion of at least 4.5 x 10^6 CD34 cells/kg may improve not only engraftment but also survival and decrease the transplant-related mortality.[23] Thus, many strategies have been investigated in order to improve the PBSC yield, including development of newer marrow mobilizing reagents targeting different adhesion pathways, combination of G-CSF with corticosteroids and/or chemotherapy reagents, and increasing blood volume processing in pediatric autologous donations.[24 25] In the present study, we focused on the donor factors that affect PBSC mobilization, hoping to find new targets for chemo intervention as well as providing information for selection of appropriate donors.

Inter-individual variety in the response to marrow mobilization has been well-documented. However, there are some controversies regarding to the effects of major factors such as age, gender, BMI on PBSC yields. In this study, we investigated healthy volunteer donors with age range from 19 years to 60 years. The strong correlation between the pre-collection CD34 count and final CD34 yield in these donors enabled us to use pre-collection CD34 as an indicator for response to mobilization which has been standardized in our facility and many other institutions. Donors with higher pre-collection CD34 counts had higher BMI. However, there was no difference in donors' age among the three responder groups. There were less female donors in the excellent responder group. But this may be due to the fact that most of the female donors had a lower BMI. Better responders had a much higher pre-collection CD34 count on a per-kg of body weight basis.

Further studies on the effect of BMI on the PBSC yield confirmed that heavier donors indeed had a better response to mobilization as suggested by a higher pre-collection CD34 per kg of body weight. And we know that the total blood volume is a direct function of body weight. Thus, heavier donors also have higher blood volume. As a result, heavier donors had better PBSC yield even with lower blood volume processing. It is important to note that low BMI donors in this study actually had the normal weight and their pre-collection CD34 counts were not low with a mean of 45/μL. However, when compared to those overweight or obese donors, their pre-collection CD34 was significantly lower.

The finding of overweight or obese adult donors who are otherwise healthy had better response to G-CSF is of interest. First, recent animal studies had demonstrated that adipose tissue contains significant number of hematopoietic stem cells. [26-28] However, the exact mechanisms determining the number of HSPCs in peripheral fat tissue are currently unknown. And it is yet to be confirmed that these hematopoietic stem cells are also present in human adipose tissue. Currently, studies have been undergoing to elucidate whether adipose tissue could be a potential source of hematopoietic stem cells.[29] Second, the G-CSF dose was determined based on donor weight. Donors with high BMI may have received higher doses of G-CSF than that if ideal body weight was used for determination of G-CSF. A previous study had shown that administering G-CSF at 16 g/kg/day leads to a higher CD34 cell yield than a dosage of 10 g/kg/day in healthy donors.[30] The efficacy of G-CSF dose as determined by the ideal body weight in obese or overweight donors remains to be investigated. Third, a decrease in G-CSF dose in obese or overweight donors may still elicit an appropriate marrow response. In this study, the pre-collection CD34 count in obese donor was close to 100/μL. A 50% reduction of pre-CD34 would be enough for most recipients since the obese donors also have a very big blood volume. This strategy could potentially reduce the unnecessary adverse effects of G-CSF which are well-known. And last, better response to G-CSF mobilization in donors with higher BMI may not be only accounted for by

relatively higher doses of G-CSF as discussed above, but also due to some other intrinsic biomedical factors that are associated with obesity. Study on the relationship between BMI and mobilization may lead to findings of new mobilizing targets that can be applied to autologous donors.

It can be concluded from our data that donor body weight or BMI is an important factor that affects PBSC yield by leukapheresis collection. High number of PBSC can be harvested from donors with high BMI. Further study on the association between donor BMI and PBSC mobilization may lead to safer, more efficient marrow mobilization.

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Table 1. Allogeneic PBSC Donor characteristics

Characteristics	
Age(years), median (range)	33 (19-59)
Gender (Male/Female)	40/29
Body weight (kg)	84 ± 2.7
Baseline WBC (x 10^3/μL)	6.6 ± 0.19
pre-Collection WBC (x 10^3/μL)	34.3 ± 1.3
pre-Collection CD34 (/μL)	71.2 ± 6.8

Figure 1

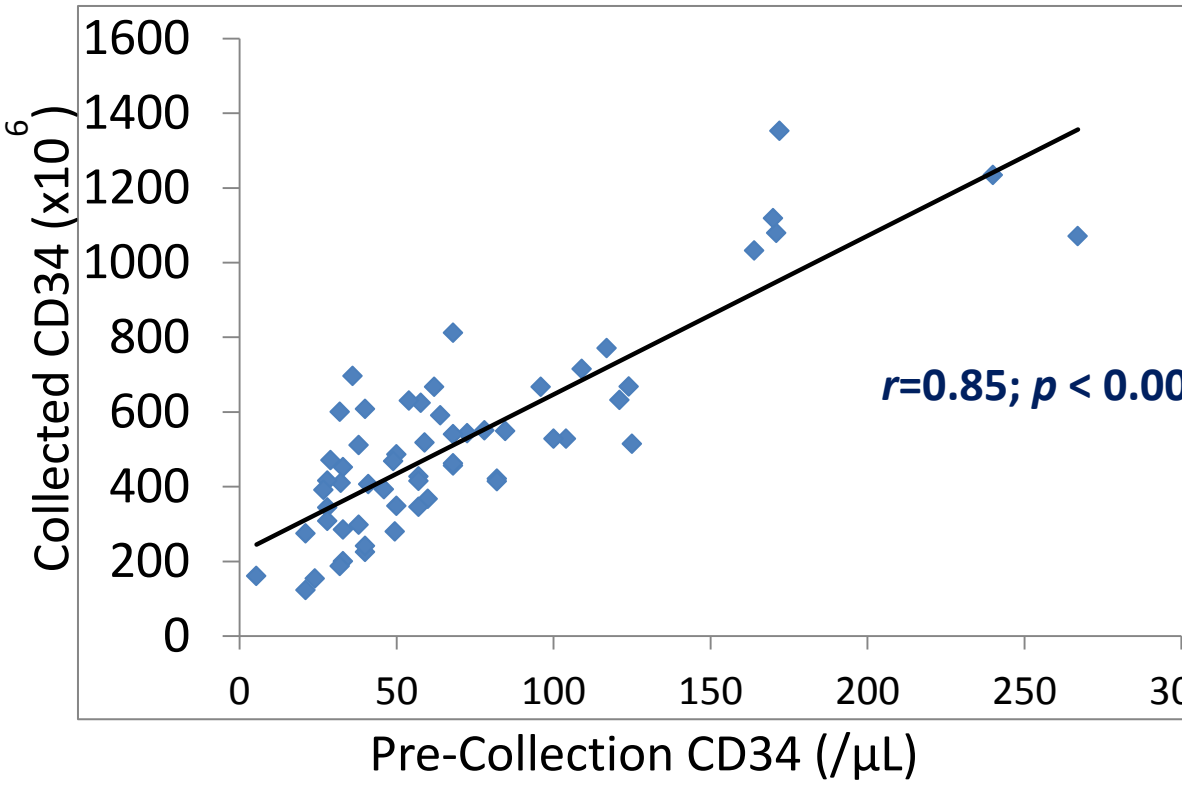
1600
1400
1200
1000
800
600
400
200
0
Collected CD34 (x10^6)
0
50
100
150
200
250
Pre-Collection CD34 (/µL)
r=0.85; p < 0.00

Figure 2

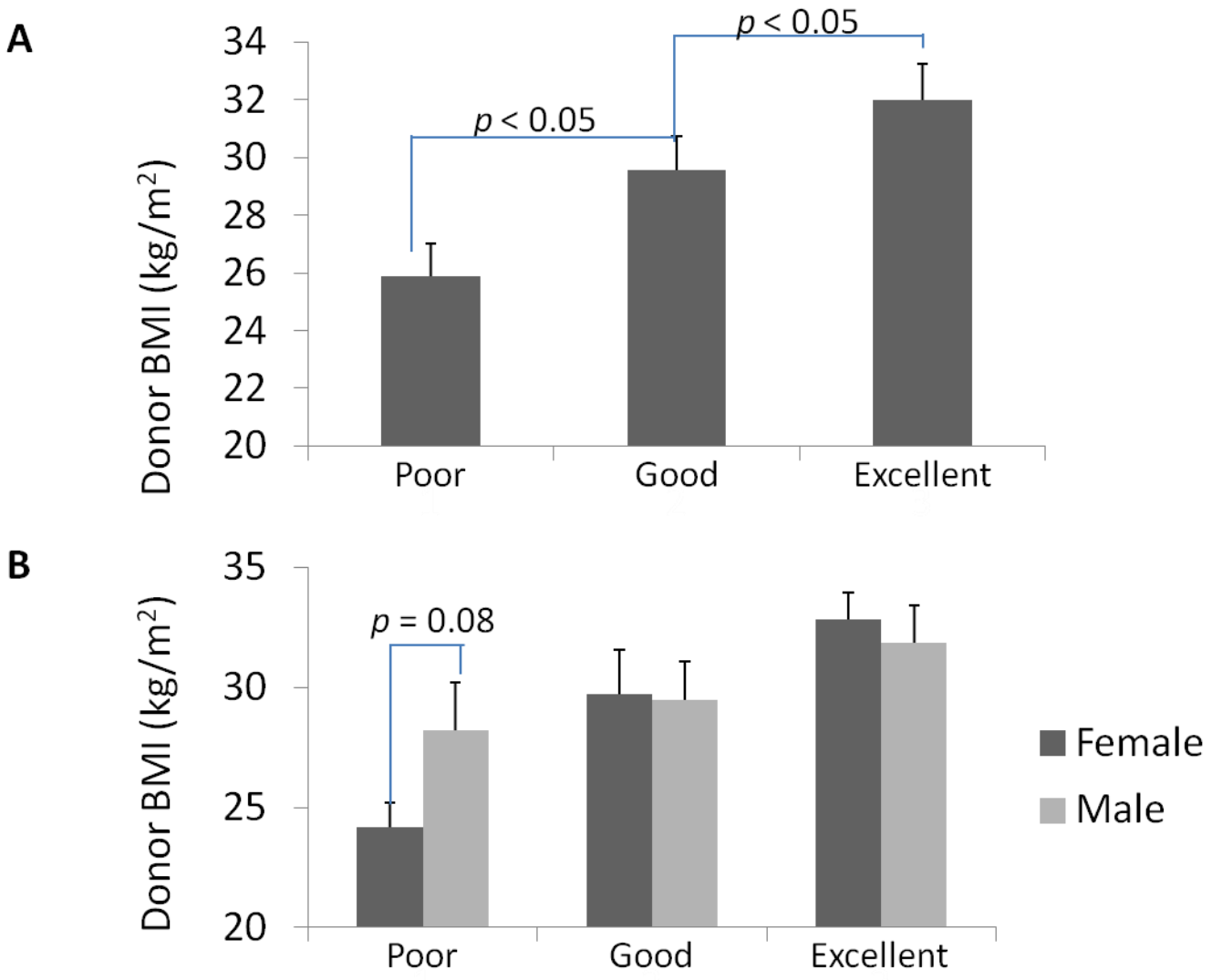
A
Donor BMI (kg/m²)
34
32
30
28
26
24
22
20
p < 0.05
p < 0.05
Poor
Good
Excellent
B
Donor BMI (kg/m²)
35
30
25
20
p = 0.08
Poor
Good
Excellent
Female
Male

Figure 3

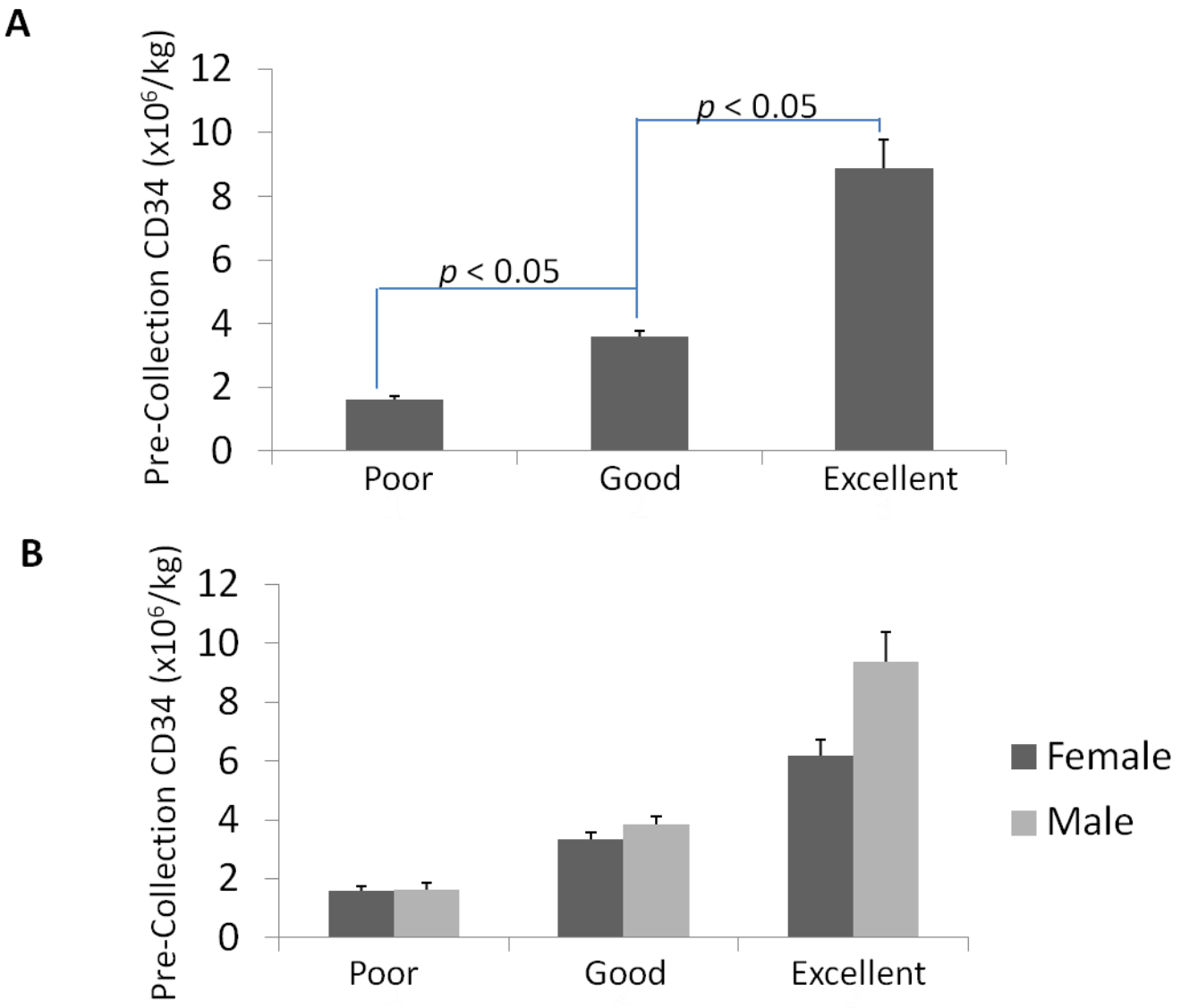
A
Pre-Collection CD34 (x10^6/kg)
12
10
8
6
4
2
0
p < 0.05
p < 0.05
Poor
Good
Excellent
B
Pre-Collection CD34 (x10^6/kg)
12
10
8
6
4
2
0
Poor
Good
Excellent
Female
Male

Figure 4

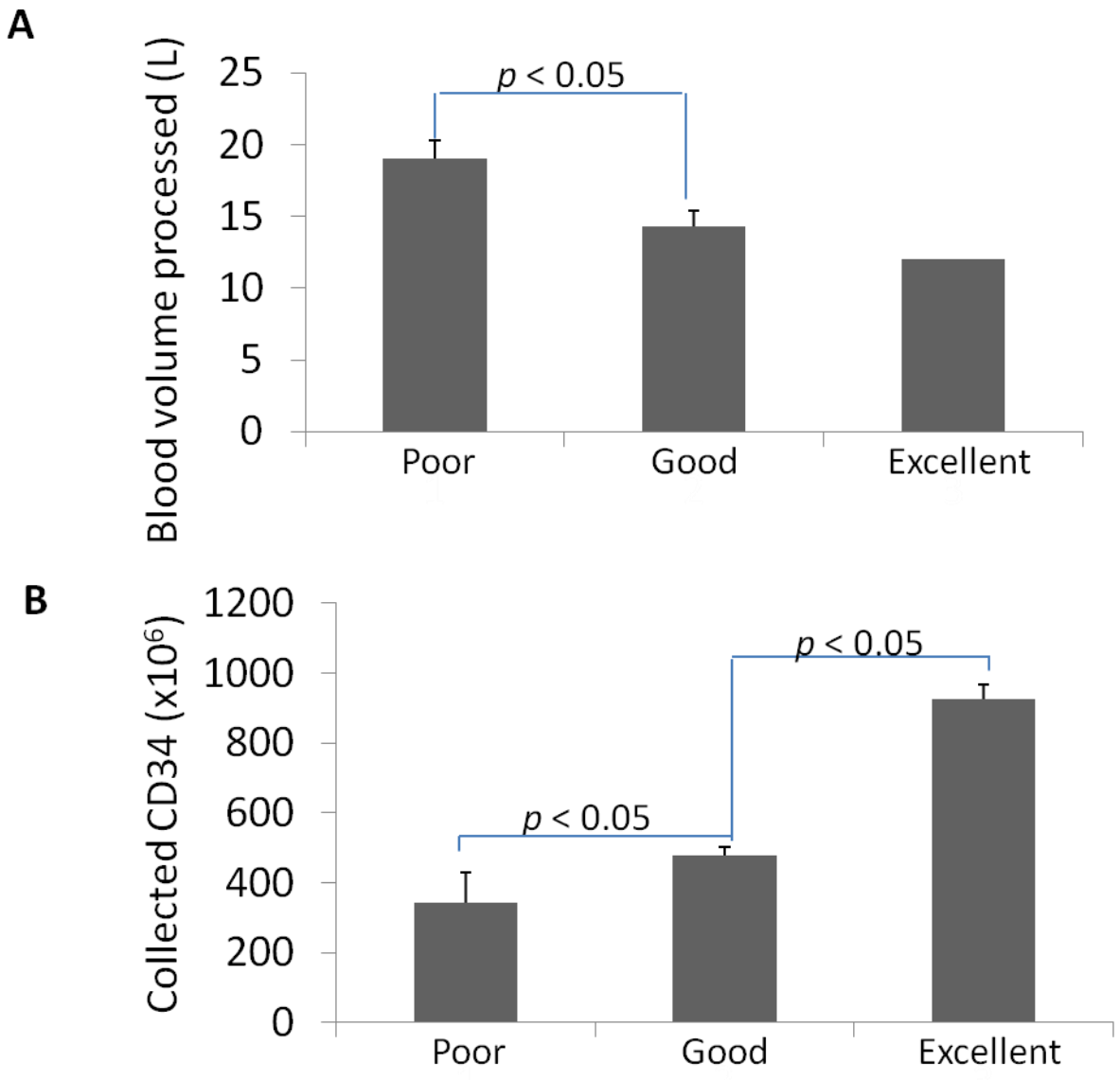
A
Blood volume processed (L)
25
20
15
10
5
0
p < 0.05
Poor
Good
Excellent
B
Collected CD34 (x10^6)
1200
1000
800
600
400
200
0
p < 0.05
p < 0.05
Poor
Good
Excellent

Figure 5

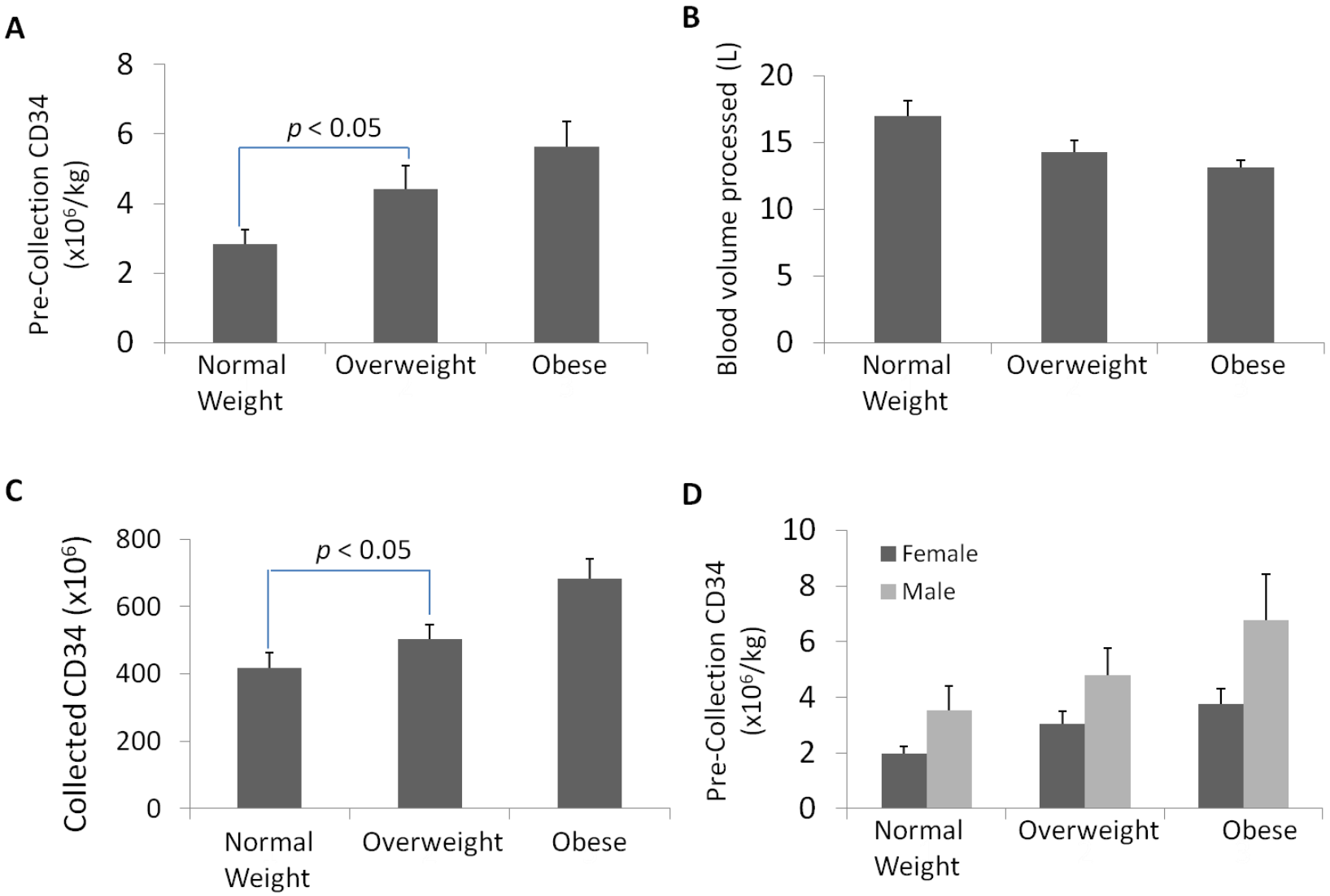
A
Pre-Collection CD34 (x10⁶/kg)
8
6
4
2
0
p < 0.05
Normal Weight
Overweight
Obese
B
Blood volume processed (L)
20
15
10
5
0
Normal Weight
Overweight
Obese
C
Collected CD34 (x10⁶)
800
600
400
200
0
p < 0.05
Normal Weight
Overweight
Obese
D
Pre-Collection CD34 (x10⁶/kg)
10
8
6
4
2
0
Female
Male
Normal Weight
Overweight
Obese

Figure legends:

Figure 1. Correlation between pre-collection CD34 count and collected CD34 cells by leukapheresis with 12 L blood processed. (n = 41)

Figure 2. Comparison of BMI between donor groups as determined by pre-collection CD34 count (A) and between female and male donors in each group (B).

Figure 3. Comparison of pre-collection CD34 count on a per kg of body weight basis between donor groups (A) and between female and male donors in each group (B).

Figure 4. Comparison of blood volume processed (A) and the amount of collected CD34 cells (B) between donor groups.

Figure 5. Effects of BMI on pre-collection CD34 count per kg of body weight (A), blood volume processed (B), and the amount of collected CD34 cells (C) after leukapheresis. Pre-collection CD34 count per kg of body weight between female and male donors with similar BMI was also compared (D).