Hemoglobin mass and blood volume change within four weeks after a blood donation.

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Abstract

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Introduction:
Acute changes of the blood volume were evaluated by primarily looking at the fluctuations of the hemoglobin concentration. A better quantitative method for the evaluation of blood volume changes could be the direct determination of the hemoglobin mass. The aim of this paper was to record the changes of the blood parameters with multiple direct blood volume determinations within four weeks of a whole blood donation of 500 ml.

Method:
The examination included ten healthy students (women n = 1; men n = 9) of the University of Leipzig. The blood volume was determined six times within five weeks using the CO-rebreathing method in the closed system. The first two measurements were taken before a whole blood donation and immediately afterwards. Four further measurements were taken at an interval of one week. The parameters hemoglobin concentration (Hb), hematocrit (HCT), blood volume (BV), erythrocytes volume (RCV) and total hemoglobin mass (tHb-mass) were determined for each measurement. Subsequently, the data was evaluated by using ANOVA with repeated measurements.

Results:
tHb-mass:
Mean value and standard deviation: T1: 948 $\pm$ 197 g; T2: 865 $\pm$ 179 g; T3: 876 $\pm$ 183 g; T4: 904 $\pm$ 179 g; T5: 919 $\pm$ 196 g; T6: 922 $\pm$ 167 g. Significant differences could be determined between the measurements T1 and T2 ($p < 0.0001$), T1 and T3 ($p < 0.0001$) and T1 and T4 ($p < 0.001$).
Blood volume:
Mean value and standard deviation: T1: 6392 $\pm$ 964 ml; T2: 5869 $\pm$ 982 ml; T3: 6180 $\pm$ 878 ml; T4: 6043 $\pm$ 1019 ml; T5: 6054 $\pm$ 969 ml; T6: 6148 $\pm$ 938 ml. A significant difference could be determined between the measurements T1 and T2 ($p < 0.001$).
Hemoglobin concentration:
Mean value and standard deviation: T1: 9.16 $\pm$ 0.97 mmol/l; T2: 9.11 $\pm$ 0.76 mmol/l; T3: 8.64 $\pm$ 0.79 mmol/l; T4: 9.25 $\pm$ 0.55 mmol/l; T5: 9.36 $\pm$ 0.82 mmol/l; T6: 9.29 $\pm$ 0.57 mmol/l. Significant differences could be determined between the measurements T3 and T4 ($p < 0.05$), T3 and T5 ($p < 0.01$) and T3 and T6 ($p < 0.05$).

Conclusion:
Four weeks after the blood donation, 69 % of the hemoglobin mass lost during blood donation could again be determined. In the case of a logistic growth curve, the complete regeneration of the Hb-mass would take about nine weeks. The blood donation intervals defined by the German Medical Association seem to be long enough to compensate the loss of hemoglobin mass from a blood donation. The hemoglobin concentration does not clearly indicate the blood loss from donating blood.

Keywords:
Blood regeneration, hemoglobin mass, blood donation, CO-rebreathing method, blood volume

Introduction:

The determination of blood volume (BV) and its components plays a prominent role in medicine in the field of transfusion medicine and traumatology but also for questions concerning performance physiology [1]. In transfusion medicine the suitability of the donor is especially based on the determination of the hemoglobin concentration to rule out a possible anemia from donating blood [2]. The variability of this concentration-dependent parameter and its inaccuracy in the early detection of anemia were proven early on [3, 4, 5, 6]. The determination of blood volume changes caused by the change of hemoglobin concentration seems to be difficult, too. A better insight into the blood system and/or the amount of oxygen transporting molecules is given by the hemoglobin mass. Some studies have already confirmed that the tHb-mass can be extremely stable over a longer period of time. The research group of Eastwood et al. (2008) [7] has monitored the hemoglobin mass in moderately physically active subjects over a period of about 100 days. The hemoglobin mass has showed very low fluctuations of about 2 % only. Because of this stability, an exact or rather direct blood volume determination using the hemoglobin mass would be more expedient than the determination with relatively variable parameters such as the hemoglobin concentration.

The CO-rebreathing method is particularly useful for repeated measurements of the hemoglobin mass what is due to its small measurement error (TEM 1.4 – 3.5 % 90 %-CI) and the fact that it is relatively harmless compared to other measurement methods for blood volume determination [8, 9, 10, 11, 12, 13, 14, 15]. Even if the mass that transports the oxygen in blood decreases by about 5 - 6 % during the examination, this method is regarded as harmless since the implemented CO-bolus corresponds to the consumption of only 6 - 7 cigarettes [15]. Furthermore, CO has a low half-life of about 135 min in the blood circulation. [15]. Based on the described problem concerning concentration-dependent parameters for the determination of the donor suitability, the protection of the donor needs to be granted by relatively stable parameters such as the hemoglobin mass. Corresponding pre-examinations could be made with the help of the CO-rebreathing method.

The aim of this paper was to ascertain the changes of the blood parameters tHb-mass, BV, RCV and Hb-concentration within four weeks of a whole blood donation and to determine the regeneration time of hemoglobin mass.

Method

Study group

The group included students of the Faculty of Sports Science of the University of Leipzig. After informing them about the procedure and risks, both orally and in written, the students had enough time for consideration. All of them gave their written informed consent for the participation on the examinations and the corresponding pre-examinations. The persons had no inflammatory diseases and no medical contraindications at the time of the examinations. After screening the subjects for inclusion and exclusion criteria in the pre-examinations, the subjects were included into the trial.

Table 1 below shows detailed information about the study group and the inclusion and exclusion criteria.

Table 1 basic data of the study group

Gender	male (n = 9)	female (n = 1)
age (years*)	25.6 ± 2.6	23
weight (kg)*	73.7 ± 9.2	81
height (cm)*	180 ± 10	179
BMI (kg/m^2)*	22.6 ± 1.2	25.28

* mean value and standard deviation of the group

Procedure

The pre-examinations included a questionnaire with questions about the general lifestyle, a resting ECG, a lung function test for the exclusion of heart and lung diseases and a bioelectrical impedance measurement to determine the right dosage of CO during the blood volume determination.

After that, the first blood volume determination was made. The following day of the initial examination was scheduled for the whole blood donation with a subsequent blood volume determination. Four further blood volume determinations followed at an interval of one week.

Figure 1 shows the time course of the examinations.

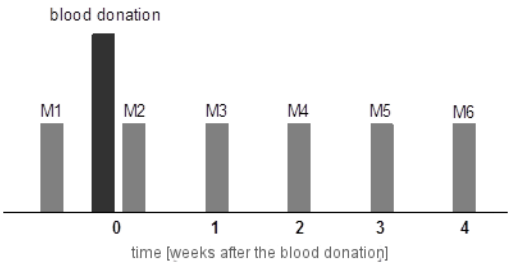

Fig. 1 study protocol (M = number of measurement)

Blood volume determination

The blood volume was measured with a CO-rebreathing method in the closed system (Falz 2013) [15].

At first, a blood gas analyzer (ABL 80 CO-OX Flex, RiliBÄK Version, Radiometer GmbH) determined the initial COHb-concentration from at least two capillary blood samples that were taken from the ear. Afterwards, the participant was connected to the closed breathing system and had a short time to get familiar with it. Then the defined CO-bolus was applied with a glass syringe. The individual bolus was defined with either 1 ml/kg body weight (men) or with 1 ml/kg fat-free mass. The subject breathed 15 minutes within this system with oxygen substitution and CO_2-absorption. Further blood samples were taken in minute 5, 9, 11, 13 and 15 to determine the COHb-concentration. After disconnecting the subject from the system, the amount of CO located in the system was determined and subtracted from the CO-bolus vol-

ume. The breathing in the closed system led to a COHb-steady-state in the vascular system from the ninth minute on. The total hemoglobin mass is calculated during the COHb-increase from the initial value until the steady-state-concentration (mean value of the values in minute 9, 11, 13 and 15).
Because of the minimal CO-loss from the vascular system, a correction factor was used (Falz 2013).

tHb-mass = K x $CO_{calculated}$ x 100 x (ΔHbCO% x 1.39)$^{-1}$

K = (relative air pressure – 14 Torr) x 760^{-1} x (1 + (0.003661 x current temperature)
$CO_{calculated}$ = CO_{adm} (ml) – $CO_{myoglobin}$ – CO_{rest} (ml)
$CO_{myoglobin}$ = CO_{adm} x 0.0023 x $t_{respiration}$ (min) (Falz 2013)
CO_{rest} = GV x CO-concentration system
GV = RV + V_{exp} + V_{system}
RV = residual volume
V_{exp} = expiratory volume
V_{system} = absorber volume + tube volume + breathing bag volume
ΔHbCO% = discrepancy between $COHb_{steady\ state}$ and $COHb_{start}$
1.39 = Hüfner's number (ml CO x gHb^{-1})

Results

Hemoglobin mass during trial

Figure 2 shows the course of the Hb-mass during the period of measurement. In total, the repeated measurements presented a highly significant change ($p < 0.0001$) in hemoglobin mass.
Viewing them individually, a significant reduction of the Hb-mass from 948 ± 197 g in the first measurement to 865 ± 179 g in the second could be observed (figure 2). This corresponds to a measured loss of 83 ± 30 g on average. The calculation of the hemoglobin loss using the hemoglobin concentration and the blood amount of 500 ml taken during the blood sample resulted in a calculated reduction of 73.75 ± 7.67 g. The difference between the calculated and measured losses clearly failed the significance limit with $p = 0.2288$.
From week three after the blood donation, there was no significant difference to the initial measurement. Especially in week one to week three after the donation, an increase of the Hb-mass could be observed. On average, the Hb-mass increased by 11.29 ± 24.94 g in week one after the blood donation, by 27.38 ± 23.65 g in week two, by 15.31 ± 33.04 g in week three and by 3.39 ± 37.74 g in week four without presenting significant changes. Table 2 shows the single measurement periods.
The post-hoc comparisons can be seen in figure 2. There were significant increases of the hemoglobin mass from M2 to M4, M2 to M5, M2 to M6, M3 to M5 and M3 to M6.

Table 2 tHb-mass

measurement	tHb-mass [g]	Difference to the initial value [g]	P
M1	948 ± 197	0	
M2	865 ± 179	83 ± 30	P < 0.0001
M3	876 ± 183	72 ± 35	P < 0.0001
M4	903 ± 179	44 ± 35	P < 0.001
M5	919 ± 196	29 ± 34	Ns
M6	922 ± 167	25 ± 32	Ns

RCV = (tHb-mass / MCHC) x 100
MCHC = mean corpuscular hemoglobin concentration (g/dl)

BV (ml) = Hb-mass (g) / Hb_{conc} (g/dl) x 100
Hb_{conc} = hemoglobin concentration (mean value from two initial measurement values)

Statistics

The statistical evaluation and graphic representation was made with the data processing programs Microsoft® Excel® 2011 for Mac (Version 14.3.9) and GraphPad Prism 6.0 (GraphPad Software Inc., California, USA). If not otherwise marked, all data were stated as mean value and standard deviation. ANOVA with repeated measurements and the Bonferroni post-hoc test were used for calculating the difference between the mean values of the measurements and the initial value. The significance limit was set as $p = 0.05$ (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

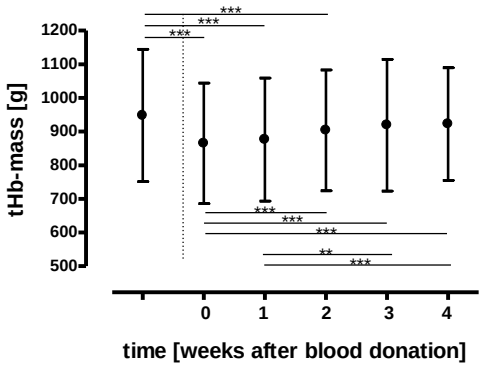

Fig. 2: Course of the tHb-mass during the trial. (MV + SD).

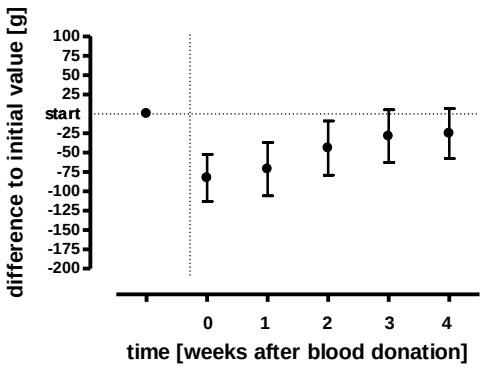

Fig. 3: tHb-mass, difference compared to the initial value. (MV + SD).

Blood volume during trial

After the blood donation, the blood volume significantly decreased parallel to the tHb-mass. Figure 4 shows the course of the blood volume during the measurement period. In total, the change of the blood volume during the trial is highly significant ($p < 0.0001$).
The initial value of the blood volume in the study group amounted to an average of 6392 ± 964 ml and

decreased significantly to 5869 ± 982 ml after the donation. According to this, the reduction amounted to an average of 523 ± 367 ml (8.29 ± 6.22 %) presenting a highly significant loss with $p < 0.001$.

The weekly repeated measurements showed no significant differences between the measurements (figure 4). Based on the described minimum after the donation, the study group had a blood volume of 6148 ± 938 ml on average in the last examination. So, four weeks after the blood donation 53% of the blood loss were compensated. This accounts to a weekly regeneration of 69 ± 187 ml on average.

From M2 to M3, the blood volume increased by 310 ± 288 ml (not significant). In the further course of measurements the blood volume remained stable (figure 4).

Measurement	BV [ml]	Difference to the initial value [ml]	P
M1	6392 ± 964	0	
M2	5869 ± 982	523 ± 367	P < 0.001
M3	6180 ± 878	213 ± 416	ns
M4	6043 ± 1019	350 ± 486	ns
M5	6054 ± 969	339 ± 523	ns
M6	6148 ± 938	245 ± 527	ns

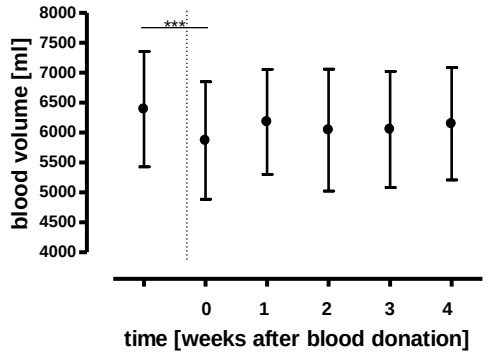

Fig. 4: Course of the blood volume during the trial. (MV + SD)

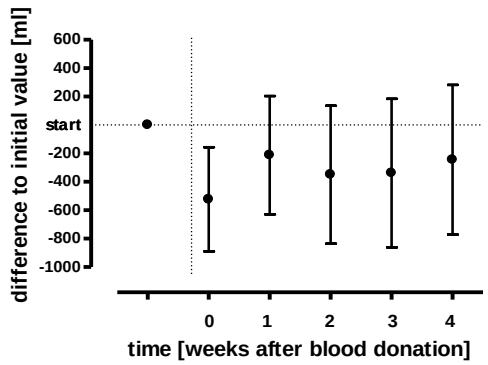

Fig. 5: blood volume, difference compared to the initial value. (MV + SD)

Hemoglobin concentration during trial

Figure 6 shows the course of the hemoglobin concentration. The change of the hemoglobin concentration was highly significant during the whole trial ($p < 0.0001$). In contrast to the Hb-mass and the blood volume, there was no change in the Hb-concentration from test one and test two (M1 9.16 ± 0.9 mmol/l; 9.11 ± 0.8 mmol/l). The lowest hemoglobin concentration with 8.64 ± 0.8 mmol/l could be observed one week after the donation. Subsequently, M4 showed that the concentration increased significantly to 9.25 ± 0.55 mmol/l. The following measurements showed that the hemoglobin concentration remained stable above the initial value (M5 9.36 ± 0.8 mmol/l; M6 9.29 ± 0.5 mmol/l). The post-hoc examinations presented significant values between the measurements M3 and M4, M3 and M5 and M3 and M6 only.

Measurement	tHb [mmol/l]	Difference to the initial value [mmol/l]	P
M1	9.16 ± 0.9	0	
M2	9.11 ± 0.7	0.04 ± 0.7	ns
M3	8.71 ± 0.8	0.44 ± 0.8	ns
M4	9.25 ± 0.5	-0.09 ± 0.9	ns
M5	9.36 ± 0.8	-0.21 ± 0.9	ns
M6	9.29 ± 0.5	-0.13 ± 0.9	ns

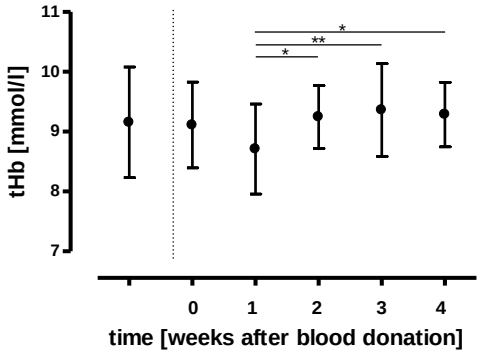

Fig. 6: Course of the hemoglobin concentration during the trial. (MV + SD)

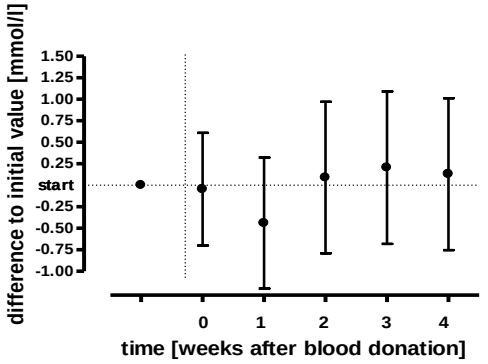

Fig. 7: Hemoglobin concentration, difference compared to the initial value. (MV + SD)

Discussion

The research demonstrated in this paper determined the changes of the directly measured parameters Hb-mass, blood volume and Hb-concentration before and after a blood donation and their subsequent regeneration within four weeks. On average, the subjects lost 8.7 ± 2.4 % of their tHb-mass during the whole blood donation. This significant reduction is comparable with earlier examinations from Specker (2009) [16], who also recognized a significant loss of 8.8 ± 1.9 %. The studies from Pottgiesser et al. (2008) [16] and Falz (2013) [15] showed similar results, too. On average, 69 % of the reduced hemoglobin mass were regenerated within four weeks of the blood donation.

Assuming a linear increase of the tHb-mass, the subjects would reach 100 % of their initial value after 6.55 ± 2.17 weeks. These results are comparable with those of Specker (2009) [16], who defined an average regeneration rate of 36 ± 11 days or 5.14 ± 1.6 weeks. Looking at the course of the tHb-regeneration, the increase seems not to be linear but forms the course of a growth curve, which declines towards the end of the measurements. Based on a logistical course, the regeneration course of the single subjects would take about nine weeks.

Parallel to Specker (2009) [16], at first, these examinations showed a low increase of the tHb-mass within the first week in comparison to the second week. Wadsworth (1955) [6], too, described similar courses in his trial. The biggest changes of the Hb-mass could be seen in week two after the blood donation.

The maturation of the erythrocytes seems to be the most possible explanation for it. The release of more EPO initiated by the Hb-loss during the blood donation is supposed to generate a first release of more reticulocytes within five days [17].

As in earlier studies, strong differences appeared in the single courses [3, 6, 16]. This resulted in regeneration times of only three weeks up to more than 11 weeks despite expecting a linear increase of the tHb-mass.

In the examination of Specker (2009) [16], one of the three female subjects showed a similar short regeneration time of only 17 days. A possible explanation for this fast regeneration could be a physical adjustment to the regular blood loss during menstruation. However, to make more detailed statements, bigger studies with more female subjects should be made. It is more likely that the strong differences in the courses were caused by measurement errors of the single measurements determining the blood volume. Assuming a possible measurement error (typical error) of the CO-rebreathing method of about 2 % [8, 9, 15], an absolute error would occur at 1000 g tHb-mass and amount to ± 20 g for each measurement.

The course of the hemoglobin concentration and hemoglobin mass were compared to show possible differences to the current determination of the donor suitability. It became apparent that there are no changes in the hemoglobin concentration after the blood donation. The reduction of the absolute hemoglobin mass after the blood donation was highly significant. At the time of the second blood volume measurement, the absolute blood loss during donation was not yet compensated by plasma volume. It was not until one week after the blood donation that a reduction of the Hb-concentration could be seen while the Hb-mass remained the same and the blood volume increased. In the next few weeks the Hb-concentration significantly increased parallel to the Hb-mass. The Hb-concentration reached even higher values after two weeks of the blood donation than at the first measurement. The total hemoglobin mass did not reach the initial values during the whole trial and, therefore, the absolute blood volume and plasma volume have to be reduced after four weeks after the donation.

The Hb-concentration does not indicate the complete regeneration of the absolute Hb-mass. This could mean that blood donors are allowed to donate again because the necessary Hb-concentration exists but the tHb-mass is not completely regenerated.

In total, the regeneration courses of the Hb-mass show that the donation intervals of 8 to 12 weeks on average set by the German Medical Association are long enough to enable an appropriate regeneration.

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