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Effects of plasmapheresis frequency on health status and exercise performance in men: A randomized controlled trial

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Abstract

Background and Objectives: Most research studies on the effects of repeated plasma donation are observational with different study limitations, resulting in high uncertainty on the link between repeated plasma donation and health consequences. Here, we prospectively investigated the safety of intensive or less intensive plasma donation protocols.

Materials and Methods: Sixty-three male subjects participated in this randomized controlled trial and were divided into low-frequency (LF, once/month, $n = 16$), high-frequency (HF, three times/month, $n = 16$), very high-frequency (VHF, two times/week, $n = 16$) and a placebo (P, once/month, $n = 15$) groups. Biochemical, haematological, clinical, physiological and exercise-related data were collected before (D0), after 1½ months (D42) and after 3 months (D84) of donation.

Results: In VHF, red blood cells, haemoglobin and haematocrit levels decreased while reticulocyte levels increased from D0 to D84. In both HF and VHF, plasma ferritin levels were lower at D42 and D84 compared to D0. In VHF, plasma levels of albumin, immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) dropped from D0 to D42 and remained lower at D84 than at D0. In HF, plasma IgG, IgA and IgM were lower at D42, and IgG and IgM were lower at D84, compared to D0. Few adverse events were reported in HF and VHF. Repeated plasma donation had no effect on blood pressure, body composition or exercise performance.

Conclusion: VHF plasmapheresis may result in a large reduction in ferritin and IgG levels. HF and VHF plasmapheresis may result in little to no difference in other biochemical, haematological, clinical, physiological and exercise-related parameters.

Keywords

albumin, donor selection, immunoglobulin, maximum oxygen consumption, maximum power output

Highlights

- This is the first randomized controlled trial prospectively investigating the effects of repeated plasma donation on a whole range of health consequences, namely biochemical and haematological parameters, blood pressure, body composition, adverse events and exercise performance.
- Haematological and biochemical parameters were severely impacted when plasmapheresis was repeated twice a week, mildly impacted with a frequency of three times per month and not impacted with a frequency of once a month. Markers for exercise performance were not altered over a 3-month period in any of the donation groups.
- Immunoglobulin G (IgG) levels dropped below the lower limit of normal (6 g/L) in the majority of donors donating twice per week. Before inducing very intensive donation regimens, the health effect of inducing hypo-IgG in donors should be investigated.

INTRODUCTION

More and more inflammatory, neurological, haematological and immunological diseases can be effectively treated with human plasma-derived products [1]. As such, the demand for plasma as the starting material for the manufacture of intravenous immunoglobulin and other plasma derivatives is growing significantly and is expected to continue to increase [2]. Future demand for intravenous immunoglobulin in developed countries is largely being driven by populations that are increasing in age and weight as well as the emergence of new indications. To increase the amount of collected plasma, one can expand the existing donor base, collect higher volumes per donation or stimulate donors to donate more frequently.

Plasma donors in the United States may donate twice within 7 days as long as the interval between donations is at least 2 days [3]. A prospective cohort study in Germany switched 3783 donors from a moderate to an intensive plasmapheresis program (maximum 60 donations annually and at least 72 h between two donations) over a 3-year period and concluded that, despite a 12.4% drop-out rate due to immunoglobulin G (IgG) levels, long-term intensive donor plasmapheresis is safe [4]. However, only very few studies have prospectively looked at the effect of repeated intensive plasma donation; either a control group was lacking [4, 5] or the control group was not randomized [6]. The majority of the studies were retrospective [7–12] or limited to one single donation [13–15]. We have recently found that repeated whole blood donation with a 3-month interval in between induced a drop in markers for iron status, which worsened with the number of donations [16]. The effects of repeated donations, whether whole blood or plasma, can be different from the effects measured after a single donation. It is therefore critical to test and document this repetitive effect to build trustable and valid guidelines concerning repetitive plasma donation. Up to now, each study has looked at a very limited number of outcomes separately, namely biochemical and haematological [4, 6, 8–11, 13, 14], blood pressure [5], clinical symptoms and adverse events [12, 13], bone metabolism [7] and exercise performance [15]. The aim of the present study was to collect data on (1) haematological and biochemical markers, (2) physiological and exercise-related parameters and (3) adverse events to get a comprehensive picture of the safety of intensive or less intensive plasma donation protocols over a 3-month period.

MATERIALS AND METHODS

Subjects

Potential study participants were either new plasmapheresis donors or previous donors who had not donated for at least 2 weeks. Eligible study participants were randomly assigned, via a computer-generated randomization table, into a placebo group (P), a low-frequency group (LF, one plasma donation per month), a high-frequency group (HF, three plasma donations per month) and a very high-frequency group (VHF, two plasma donations per week) to participate in this longitudinal study. The donation frequency regimen in the VHF group (two times/week) represents the current plasma donation regulation in countries such as Austria, Germany, Hungary, the United States, and Canada, whereas most other countries have a minimum donation interval of 14 days [17]. The P group donated at the same frequency as LF (once/month). All participants were blinded to the group selection.

Inclusion criteria were as follows: male, age 18–50 years, body mass index (BMI) 20–28 kg/m^2, and no contraindication to perform maximum-intensity exercise assessed by the physical activity readiness questionnaire. During the whole duration of the study, subjects were asked to maintain their habitual lifestyle, that is, physical activity and diet. All participants provided written informed consent after being explained about all potential risks of the study and the right to withdraw from it at any time. This study was conducted at UCLouvain, Belgium, from March 2022 to December 2022, and was approved by the Ethics Committee of UCLouvain (2020/04NOV/541). The investigation was performed according to the principles outlined in the Declaration of Helsinki. The study (RK2020) was registered at clinicaltrials.gov and received the identifier NCT05815615. Participants and the public were not involved in the research other than by their participation in the study.

Experimental procedures

One week before the first plasma donation (D0, Visit 1), subjects reported to the exercise physiology laboratory. First, systolic and diastolic blood pressure (SBP and DBP) were measured automatically (Omron) in the supine position. Then, five blood samples were taken

from an antecubital vein from the non-dominant arm: three 4-mL EDTA tubes, one 8-mL clot activator tube and one 4-mL sodium fluoride/potassium oxalate tube. After blood sampling, a maximum strength test of the dominant arm was performed using an electronic dynamometer (Grip-D, Takei, Japan) followed by a maximum strength test (1RM) with the dominant leg on a leg extension machine (ProDual, Body-Solid, IL, USA). After an individualized and standardized warm-up protocol, the 1RM test consisted of a maximum of five attempts interspersed by 3 min of rest. The 1RM corresponded to the highest load lifted once with a correct technique. Then, body mass and height were measured (Seca GmbH, Hamburg, Germany) and body composition (bone mineral content, fat-free mass and fat mass) was assessed by dual-energy X-ray absorptiometry scan (Discovery W, Hologic Inc., MA, USA). Finally, a progressive test on a bicycle ergometer (Cyclus 2, RBM elektronik automation GmbH, Leipzig, Germany) was performed to measure the peak oxygen consumption (VO_2 peak). The test started at 70 W, followed by incremental loads of 30 W every 2 min until exhaustion. The maximum power output (P_{max}) was calculated as the last step completed plus the last increment corrected for the sustained duration, which corresponded to the total time of the test. VO_2, carbon dioxide production (VCO_2) (Medisoft Ergocard, MGC Diagnostics Corporation, MN, USA) and the heart rate (HR) (Polar, Kempele, Finland) were continuously monitored during the test. Pulse oxygen was calculated by dividing VO_2 peak and HRmax at the end of the test. Blood lactate was measured before, during (at 190 W) and at the end of the test by taking a capillary blood sample (5 µL) from an earlobe (Lactate Pro, Arkray, Japan). The whole sampling and testing procedure was repeated 42 days (D42, Visit 2) and 84 days (D84, Visit 3) after the first plasma donation under exactly the same conditions. Adverse events, categorized according to international standards [18], were recorded in the blood information system throughout the entire experiment. Citrate reactions were not considered in our analysis because preventive calcium supplements were provided routinely to the regular donors.

Plasma donation

One week after blood sampling and exercise pre-testing (D0 or Visit 1), participants reported to the Red Cross Center in Leuven or Mechelen (Belgium). They underwent a plasma donation of maximum 650 mL (exclusive of anticoagulant) according to the Belgian Law of 01/02/2005, without exceeding 20% of total body volume during or 16% of total body volume at the end of the plasma donation (donation group), or had a similar sensation of undergoing a plasma donation (P group), by infusing NaCl 0.9% using a NexSys PCS device (Haemonetics). During each donation or simulation of donation, the punctured arm was shielded, and subjects were listening to music through a headset. According to our standard operating procedures, a rinse back with NaCl 0.9% (34 or 50 mL) was given after each cycle and at the end of the plasmapheresis procedure. In total, a volume of 30 mL of whole blood (six samples) was collected at each donation.

Blood analyses

Each tube was centrifuged for 10 min at 2000*g* at 4°C and analysed within 24 h following the blood drawing. The supernatant was collected and stored at −80°C. The following parameters were analysed in a medical analysis laboratory (LIMS MBnext Group Europe, LLN, Belgium): red blood cells, haemoglobin, haematocrit, reticulocyte, iron, ferritin, C-reactive protein (CRP), glycaemia, insulinemia, glycated haemoglobin (HbA1c), creatine kinase (CK), total cholesterol, albumin, immunoglobulin A (IgA), IgG and immunoglobulin M (IgM).

Statistical analysis

A statistical power analysis was carried out to determine the optimum number of subjects needed to find a difference in total serum protein mean of 10% [8] with a standard deviation of 8% and a power of 80% according to the calculator developed by Wang and Ji [19]. According to this analysis, a total of 64 subjects were needed to participate in the study to reach an optimum number of 16 subjects per group. Potential differences in the subjects' characteristics at baseline were analysed with one-way analysis of variance (ANOVA) (IBM SPSS Statistics, Version 28.0, Armonk, NY, USA). A mixed ANOVA model for repeated measures (SAS Statistical Software 9.4, SAS Institute, Cary, NC, USA) was used with the subjects as the random variable and groups (P and donations) and condition (time) as fixed independent variables. The *p*-values of the main effects can be found in Table S1. The model used the Kenward–Roger approximation of the degree of freedom with compound symmetry variance-covariance structure. When appropriate, contrast analyses were performed to compare means, applying a Sidak correction. Linear mixed models for repeated measures give unbiased results in the presence of missing data and take potential differences at baseline into account. Normality of residuals was tested using a *Q*–*Q* plot. The total number of adverse events and the adverse event rate (per 50 donations) were calculated. Statistical significance was set at $p < 0.05$. All values are expressed as mean ± SEM, except donation history which is reported as a median (interquartile range).

RESULTS

Subject characteristics

Seventy-two volunteers were enrolled in the study: 17 in P, 16 in LF, 20 in HF and 19 in VHF groups (Figure 1). Nine subjects (two in the P group, four in the HF group and three in the VHF group) withdrew before the end of the study due to personal reasons or impossibility to comply with the repeated appointments. At the time of withdrawal, IgG (9.9 ± 1.0 g/L), Hb (14.1 ± 0.4 g/dl) and ferritin levels (64 ± 15 µg/L) were not different in this group of nine subjects compared to the other subjects. Only one subject in the VHF group presented low IgG levels (4.9 g/L) at the time of withdrawal. As they

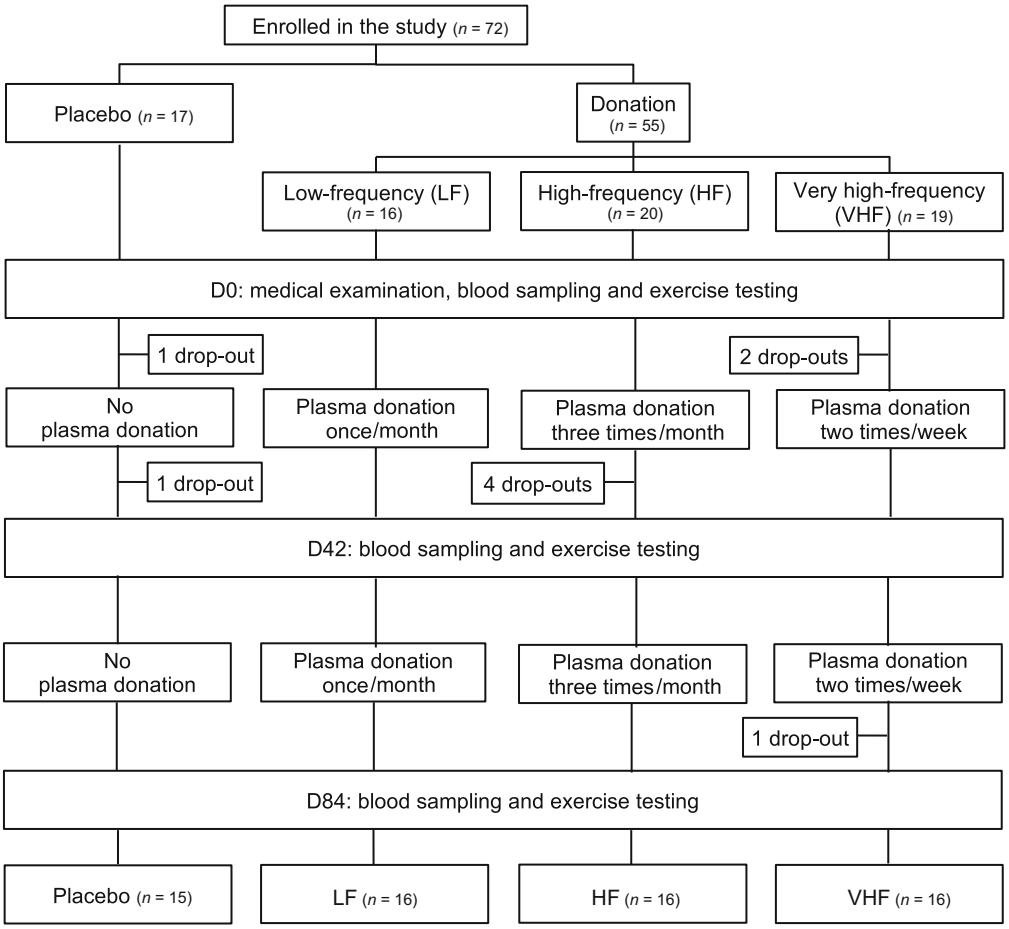

FIGURE 1 Subjects flow-chart.

were incomplete, the data of drop-outs were not included in the analyses, resulting in a total of 63 subjects: 15 in P, 16 in LF, 16 in HF and 16 in VHF groups. All participants were regular donors, except one new donor (who was allocated to the LF group). At the start of the study, the four groups were not different regarding age, BMI, the amount of physical activity per week, VO_2 peak and plasma donation history (Table S2). In addition, the recent history of whole blood donations was not different among the study groups—4 out of 16 donors in the VHF group, 3 out of 16 donors in the LF and HF groups and 3 out of 15 donors in the P donated whole blood once in the 3 months before entering the study.

Forty-nine study participants were 100% compliant with the corresponding donor regimen (5 [31%] in the VHF group, 14 [88%] in the HF group, 15 [94%] in the LF group and 15 [100%] in the P group). In the VHF group, six participants missed 1 donation, two participants missed 2–3 donations, two participants missed 5–6 donations and one participant missed 10 donations. Donors with missed donations were included in the data analysis.

VHF donation affects ferritin levels

In VHF, red blood cells ($p < 0.001$), Hb ($p < 0.001$) and haematocrit ($p = 0.003$) levels decreased, whereas reticulocyte levels ($p < 0.001$) increased from D0 to D84 (Table 1 and Figure 2a,b). In addition, reticulocyte levels were higher at D42 compared to D0 ($p < 0.001$). In both HF and VHF, plasma ferritin levels were lower at D42 ($p = 0.039$ in HF; $p = 0.001$ in VHF) and at D84 ($p = 0.008$ in HF; $p < 0.001$ in VHF) compared to D0. Except for red blood cells, all aforementioned effects of repeated plasma donation in HF and VHF were different from those of P at the same time point.

The reduced ferritin levels in the VHF (from 50.2 [D0] to 20.1 μg/L [D84], 60% reduction) were considered to be clinically meaningful. The other statistically significant differences were of no clinical importance.

VHF donation affects IgG levels

In VHF, the plasma levels of albumin (Figure 2c), IgG (Figure 2d), IgA and IgM dropped substantially from D0 to D42 ($p < 0.001$) and remained lower at D84 than at D0 ($p < 0.001$) (Table 1). Albumin and IgG levels at D42 and D84 in VHF were lower than those of P at the same time ($p < 0.01$–0.001). In HF, compared to D0, plasma IgG ($p < 0.001$), IgA ($p = 0.011$) and IgM ($p = 0.006$) were lower at D42, and IgG ($p < 0.001$) and IgM ($p < 0.001$) lower at D84. CRP, CK, SBP and DBP were unaffected by repeated plasma donation (data not shown).

TABLE 1 Effects of repeated plasma donation on haematological parameters.

		D0	D42	D84
RBC (10^6/mm^3)	P	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1$^{###}$
	LF	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
	HF	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
	VHF	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.01$^{###}$
Haematocrit (%)	P	41.8 ± 0.7	42.3 ± 0.7	43.5 ± 0.7$^{###}$
	LF	42.0 ± 0.3	42.1 ± 0.3	42.0 ± 0.5
	HF	42.3 ± 0.6	42.3 ± 0.7	43.2 ± 0.5
	VHF	42.1 ± 0.4	41.8 ± 0.7	40.6 ± 0.4$^{##,§§§}$
Reticulocytes (%)	P	1.3 ± 0.1$^{£}$	1.4 ± 0.1	1.3 ± 0.1
	LF	1.3 ± 0.1$^{£}$	1.3 ± 0.1	1.3 ± 0.1
	HF	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1§
	VHF	1.3 ± 0.1$^{£}$	1.6 ± 0.1$^{###,§}$	1.6 ± 0.1$^{###,§}$
Iron (μg/dL)	P	109.3 ± 12.0	109.8 ± 12.3	122.7 ± 13.9
	LF	106.8 ± 10.2	100.3 ± 7.4	103.3 ± 7.1
	HF	83.6 ± 10.1	106.4 ± 10.6	91.3 ± 9.6§
	VHF	75.7 ± 10.0§,}$	78.8 ± 9.1§	65.0 ± 8.2§§§
Glycaemia (mg/dL)	P	92.5 ± 3.5	82.3 ± 5.3	91.4 ± 4.6
	LF	91.6 ± 4.2	91.9 ± 3.4	92.4 ± 5.6
	HF	94.9 ± 3.1	94.2 ± 4.2§	78.6 ± 3.7$^{##,§}$
	VHF	90.6 ± 2.4	82.4 ± 3.5$^{£}$	79.9 ± 3.6$^{#,§}$
Insulinemia (pmol/L)	P	73.5 ± 12.2	89.6 ± 16.4	92.6 ± 14.7
	LF	76.3 ± 17.2	83.1 ± 12.7	98.6 ± 15.0
	HF	76.8 ± 15.9	138.2 ± 31.4$^{#}$	87.0 ± 18.3
	VHF	77.5 ± 15.7	133.4 ± 47.0$^{#}$	114.9 ± 29.9
HbA1c (%)	P	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
	LF	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
	HF	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
	VHF	5.3 ± 0.1	5.2 ± 0.1$^{#}$	5.1 ± 0.1$^{###}$
Total cholesterol (mg/dL)	P	162.7 ± 10.3	167.1 ± 8.9	175.9 ± 8.2$^{#}$
	LF	161.3 ± 7.2	167.9 ± 7.3	171.4 ± 8.9$^{#}$
	HF	160.5 ± 5.6	180.6 ± 8.2$^{###}$	175.2 ± 8.1$^{##}$
	VHF	182.9 ± 10.9	188.4 ± 12.5	189.4 ± 10.8
IgA (g/L)	P	2.2 ± 0.2	2.4 ± 0.3	2.4 ± 0.3
	LF	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2
	HF	2.2 ± 0.2	1.9 ± 0.1$^{#}$	2.1 ± 0.2
	VHF	2.5 ± 0.3	2.0 ± 0.2$^{###}$	2.1 ± 0.2$^{###}$
IgM (g/L)	P	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
	LF	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1$^{#}$
	HF	0.9 ± 0.1	0.8 ± 0.1$^{##}$	0.7 ± 0.1$^{###}$
	VHF	0.9 ± 0.1	0.6 ± 0.1$^{###}$	0.7 ± 0.1$^{###}$

Note: Values are means ± SEM. $n = 15$ in the P group, $n = 16$ in LF, HF and VHF groups.
Abbreviations: HbA1c, glycated haemoglobin; HF, high-frequency; IgA, immunoglobulin A; IgM, immunoglobulin M; LF, low-frequency; P, placebo; RBC, red blood cells; VHF, very high-frequency.
$^{#}p < 0.05$; $^{##}p < 0.01$ and $^{###}p < 0.001$ different from D0, same group. $^{§}p < 0.05$; $^{§§§}p < 0.001$ different from P, same time.

The reduced IgG levels in the VHF group (from 9.23 [D0] to 5.73 g/L [D84], 38% reduction, 9 out of 16 [56%] individuals with IgG levels <6 g/L at D84) were considered to be clinically meaningful. The other statistically significant differences were within the normal ranges and of no clinical importance.

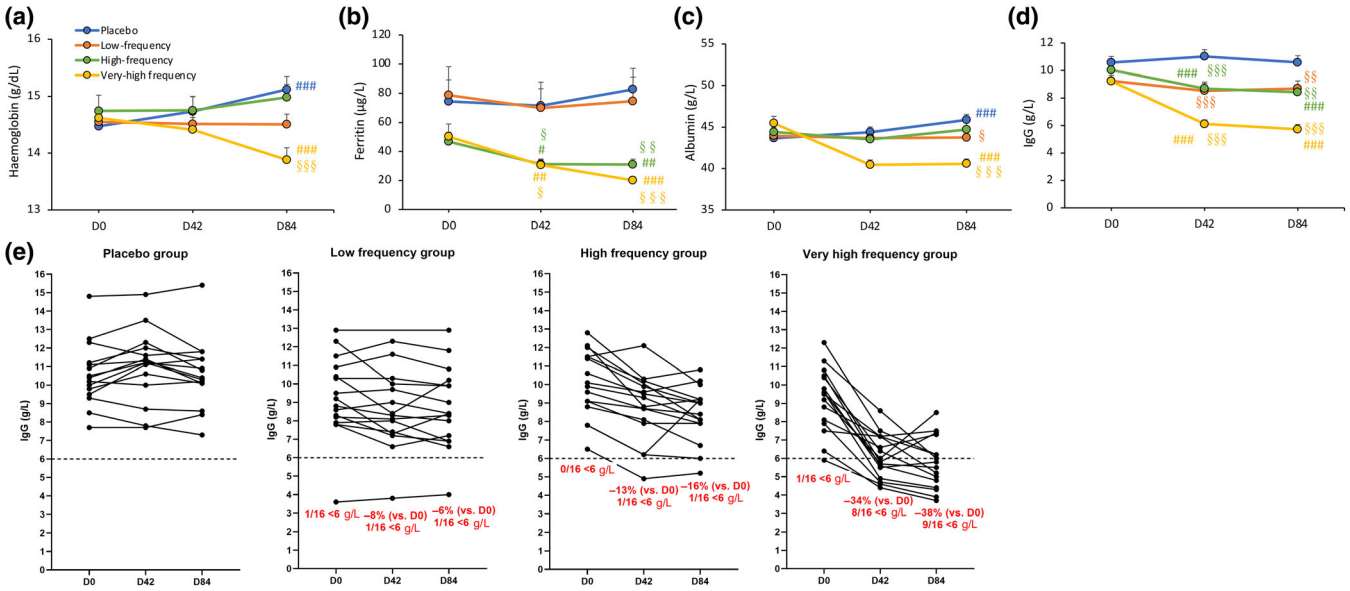

FIGURE 2 Plasma haemoglobin, ferritin, albumin and immunoglobulin levels. Evolution of plasma haemoglobin (a), ferritin (b), albumin (c), mean immunoglobulin G (IgG) (d) and individual IgG (e) levels before (D0), during (D42) and after (D84) the 3-month trial in the placebo (P) and donation groups. Data are expressed as means ± SEM. $n = 15$ in the placebo group and $n = 16$ in each of the three donation groups. $^{\#}p$ <0.05; $^{\#\#}p$ <0.01 and $^{\#\#\#}p$ <0.001 different from D0, same group. $^{\S}p$ <0.05; $^{\S\S}p$ <0.01 and $^{\S\S\S}p$ <0.001 different from P, same time.

No effect of repeated plasma donation on glycaemia, insulinemia and cholesterol levels

Glycaemia decreased from D0 to D84 in the HF ($p = 0.002$) and VHF ($p = 0.042$) groups and was lower in both at D84 compared to P (p <0.05) (Table 1). Insulinemia increased from D0 to D42 in HF ($p = 0.035$) and VHF ($p = 0.019$). In VHF, plasma HbA1c levels decreased from D0 to D42 ($p = 0.007$). Compared to D0, total cholesterol levels were higher at D42 in HF ($p < 0.001$) and at D84 in P ($p = 0.014$), LF ($p = 0.049$) and HF ($p = 0.005$). These differences were of no clinical importance.

No effect of repeated plasma donation on body composition

Fat-free mass and fat-free mass + bone mineral content were lower at D84 compared to D0 ($p = 0.040$ and $p = 0.049$, respectively) in P (Table 2). Fat mass increased from D0 to D84 in HF ($p = 0.038$) and VHF ($p = 0.041$). No effect was found for body mass, BMI or bone mineral content.

No effect of repeated plasma donation on exercise performance

The maximum power output decreased from D0 to D42 in LF ($p = 0.019$) and from D0 to D42 ($p = 0.013$) and D84 ($p = 0.012$) in VHF (Figure 3a). VO_2 peak was higher at D42 compared to D0 in HF ($p = 0.009$, Figure 3b). Pulse oxygen was higher at D42 in P ($p = 0.037$) and HF ($p = 0.006$) and higher at D84 ($p = 0.027$) in LF compared to D0 (Table 3). No effect was found for lactate at 190 W, lactate post exercise, maximum ventilation, maximum heart rate, maximum quadriceps (Figure 3c) and arm strength.

Few clinical adverse events were reported in the HF and VHF groups

The occurrence of clinical adverse events was monitored in each group during the whole experimental trial (Table S3). Five haematomas were present in HF (three events in three donors, adverse event rate: 1.08) and VHF (two events in one donor, adverse event rate: 0.28). Five vasovagal reactions were reported, one in HF (one donor, adverse event rate: 0.36) and four in VHF (three donors, adverse event rate: 0.57). Five anaemia events, defined as a Hb level below 135 g/L, were detected in four VHF donors (adverse event rate: 0.71). All anaemic participants had ferritin levels below or equal to 50 µg/L at D0. No other (major) events were reported.

DISCUSSION

For the first time, data on (1) haematological and biochemical markers, (2) physiological and exercise-related parameters and (3) adverse events were prospectively collected over 3 months to get a comprehensive picture of the health consequences of intensive or less intensive plasma donation protocols. We found that repeated plasma donation induced (1) a large reduction in ferritin and IgG levels in the

TABLE 2 Effects of repeated plasma donation on body composition.

		D0	D42	D84
Body mass (kg)	P	77.4 ± 3.8	77.7 ± 3.8	77.2 ± 3.7
	LF	78.9 ± 2.6	79.1 ± 2.7	79.5 ± 2.5
	HF	82.8 ± 2.1	83.7 ± 2.5	83.5 ± 2.5
	VHF	80.7 ± 2.9	80.2 ± 2.8	80.7 ± 3.0
BMI (kg/m^2)	P	23.6 ± 0.9	23.7 ± 0.9	23.6 ± 0.9
	LF	23.7 ± 0.6	23.6 ± 0.6	23.8 ± 0.7
	HF	24.4 ± 0.7	24.7 ± 0.8	24.7 ± 0.8
	VHF	24.0 ± 0.6	23.8 ± 0.5	24.0 ± 0.6
BMC (kg)	P	2.87 ± 0.12	2.88 ± 0.12	2.91 ± 0.12
	LF	2.97 ± 0.13	2.95 ± 0.13	2.98 ± 0.13
	HF	2.95 ± 0.06	2.95 ± 0.05	2.93 ± 0.06
	VHF	2.89 ± 0.11	2.87 ± 0.11	2.88 ± 0.11
Fat mass (kg)	P	12.7 ± 1.9	12.4 ± 1.8	13.3 ± 1.9
	LF	11.5 ± 1.1	11.5 ± 1.1	11.8 ± 1.1
	HF	14.6 ± 1.4	15.1 ± 1.5	15.4 ± 1.5$^{\#}$
	VHF	14.1 ± 1.3	13.7 ± 1.3	14.9 ± 1.4$^{\#}$
FFM (kg)	P	60.6 ± 2.3	60.9 ± 2.2	59.8 ± 2.1$^{\#}$
	LF	63.2 ± 1.7	63.2 ± 1.8	63.2 ± 1.7
	HF	63.9 ± 1.2	64.2 ± 1.2	63.6 ± 1.2
	VHF	61.8 ± 2.0	61.9 ± 1.8	61.5 ± 1.9
FFM + BMC (kg)	P	63.5 ± 2.4	63.8 ± 2.3	62.7 ± 2.2$^{\#}$
	LF	66.1 ± 1.8	66.1 ± 1.9	66.2 ± 1.7
	HF	66.8 ± 1.2	67.2 ± 1.2	66.6 ± 1.2
	VHF	64.7 ± 2.1	64.8 ± 1.9	64.3 ± 1.9
% Fat	P	15.9 ± 1.6	15.5 ± 1.6	16.8 ± 1.6
	LF	14.6 ± 1.0	14.5 ± 1.0	14.9 ± 1.0
	HF	17.5 ± 1.3	17.9 ± 1.4	18.3 ± 1.4
	VHF	17.7 ± 1.2	17.1 ± 1.2	18.4 ± 1.2

Note: Values are means ± SEM. $n = 15$ in the P group, $n = 16$ in LF, HF and VHF groups.
Abbreviations: BMC, bone mineral content; BMI, body mass index; FFM, fat-free mass; HF, high-frequency; LF, low-frequency; P, placebo; VHF, very high-frequency.
$^{\#}p$ <0.05 versus D0, same group.

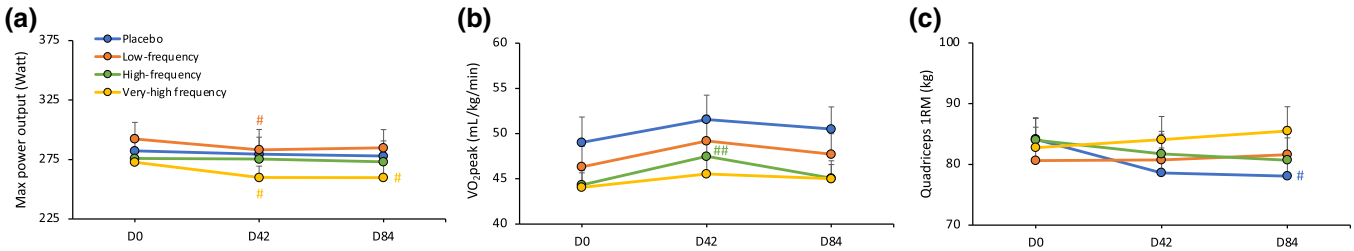

FIGURE 3 Markers for endurance and strength performance. Evolution of the maximum power output (a), peak oxygen consumption (VO_2 peak) (b) and maximum strength of the quadriceps (1RM) (c) before (D0), during (D42) and after (D84) the 3-month trial in the placebo and donation groups. Data are expressed as means ± SEM. $n = 15$ in the Placebo group, and $n = 16$ in each of the three donation groups. $^{\#}p$ <0.05, $^{\#\#}p$ <0.01 different from D0, same group.

VHF group; (2) a few minor clinical adverse events in both the HF and VHF group; (3) little to no difference in other biochemical, haematological, physiological and exercise-related parameters.

This is the first randomized controlled trial prospectively investigating the health consequences of repeated plasma donation. Most previous studies in this domain had an observational study design,

TABLE 3 Effects of repeated plasma donation on exercise performance.

		D0	D42	D84
Lactate 190 W (mmol/L)	P	3.5 ± 0.5	3.5 ± 0.5	3.3 ± 0.4
	LF	2.9 ± 0.5	2.8 ± 0.6	3.1 ± 0.6
	HF	3.0 ± 0.2	3.2 ± 0.4	3.1 ± 0.3
	VHF	3.9 ± 0.8	3.8 ± 0.6	3.3 ± 0.4
Lactate post (mmol/L)	P	11.2 ± 0.7	10.2 ± 0.7	10.1 ± 0.8
	LF	9.4 ± 0.7	9.3 ± 0.7	9.4 ± 0.5
	HF	10.1 ± 0.8	9.8 ± 0.6	9.6 ± 0.7
	VHF	11.6 ± 0.5	11.1 ± 0.4	10.9 ± 0.5
HRmax (bpm)	P	185 ± 3	185 ± 2	185 ± 3
	LF	180 ± 5	181 ± 5	180 ± 5
	HF	184 ± 4	185 ± 3	184 ± 3
	VHF	188 ± 2	189 ± 2	189 ± 2
VEmax (L/min)	P	150 ± 8	150 ± 9	145 ± 8
	LF	141 ± 7	145 ± 7	144 ± 9
	HF	145 ± 3	148 ± 4	146 ± 4
	VHF	142 ± 7	140 ± 6	140 ± 5
Oxygen pulse (mL/beat·kg)	P	0.20 ± 0.01	0.21 ± 0.01$^{\#}$	0.21 ± 0.01
	LF	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01$^{\#}$
	HF	0.20 ± 0.01	0.21 ± 0.03$^{\#\#}$	0.20 ± 0.03
	VHF	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Max arm strength (kg)	P	43 ± 2	43 ± 2	45 ± 2
	LF	44 ± 2	45 ± 2	45 ± 3
	HF	41 ± 1	41 ± 2	41 ± 1
	VHF	44 ± 2	43 ± 2	43 ± 2

Note: Values are means ± SEM. $n = 15$ in the P group, $n = 16$ in the LF, HF and VHF groups.
Abbreviations: HF, high-frequency; HR, heart rate; LF, low-frequency; P, placebo; VE, ventilation; VHF, very high-frequency.
$^{\#}p$ <0.05; $^{\#\#}p$ <0.01 versus D0, same group.

with different study limitation (e.g., not controlled for confounding), resulting in high uncertainty on the (causal) link between repeated plasma donation and health consequences [20–23]. One previous non-randomized controlled study compared total serum protein, albumin, IgG, IgA and IgM levels after weekly or bi-weekly (>14 days) plasmapheresis for 6 months to levels obtained in regular blood donors [6]. This study showed that total protein and IgG levels in the weekly group were lower than those in the control and the bi-weekly groups, but remained well within the normal ranges. Albumin, IgA and IgM levels were not modified by plasmapheresis. Our HF group, corresponding more or less to the weekly group in Ciszewski et al., had lower plasma ferritin, IgG, IgA and IgM levels 3 months after plasma donation. Our VHF group was even more impacted, with Hb and albumin levels being down-regulated as well compared to the start of plasma donation and compared to the P group. Except for IgG and ferritin in the VHF group, the drop in all other haematological and biochemical parameters in the HF and/or VHF group did not cross the lower acceptable limits, on average. Of note, before the start of the study, IgG levels were slightly lower in the LF and VHF groups compared to the P group but still well within physiological range. All studies that investigated IgG found reduced levels in donors with frequencies from once a month to twice a week, with a higher risk at falling below the normal range [4, 6, 8, 11]. Here, we found that IgG levels were reduced even in the LF group, donating once a month, while IgA and IgM levels were decreased only in the HF and VHF groups. IgG levels dropped below the lower limit of normal in the majority of donors in the VHF group. In the absence of solid evidence demonstrating that induced hypo-IgG is harmless for the donor, VHF donation regimens are not in line with the precautionary principle to avoid harm to the donor. Although Belgian plasma donors donate, on average, only 4–5 times per year, more than 50% of plasma-derived IgG administered to patients in Belgian hospitals originates from Belgian donors, indicating that VHF donation regimens are not essential to obtain self-sufficiency.

Lower ferritin values were previously reported in frequent plasma donors compared to non-donors [9, 11], with its levels being negatively correlated to the number of donations per year [9]. When looking at individual values for ferritin levels, 5 volunteers out of 16 in the VHF group had values <12 μg/L at the end of 3 months. Those results contrast with those of a previous study that retrospectively looked at

ferritin levels over a period of 12 months, during which plasma was donated at frequencies ranging from 0 to more than 70 times [10]. Less than 1% of male donors presented ferritin levels below 12 μg/L even in the group donating at least 70 times over 12 months, approaching the frequency in our VHF group. Divergent results have been reported as well concerning Hb levels, with one study reporting lower levels in frequent plasma donors [9] and another finding no effect [11] even when donating at least once a week for 12 months. In donors donating plasma several times per month, strategies to reduce the loss of red blood cells, such as rinsing back at the end of the procedure, limiting the number of whole blood samples and reducing the number of procedural failures resulting in incomplete return of red cells to the donor, should be considered and tested for their effectiveness.

Alongside determining the effects of repeated plasma donation on haematological and biochemical markers, the second aim of the study was to investigate the functional and physiological impact of repeated donation. We found no effect of 3-month plasma donation on blood pressure, body mass, body composition, markers for endurance performance and maximum strength. SBP and DBP were found to be decreased after 4-month plasma donation at intervals of less than 14 days in donors with high baseline blood pressure levels [5]. Here, in donors with normal blood pressure levels at the start of the study, no change of blood pressure was observed, even in the most intensive groups. Based on the decrease in some haematological parameters and a previous study looking at the effect of one single plasma donation on exercise performance [15], we would have expected a decrease in markers for endurance performance in the VHF group. The maximum power output, maximum oxygen consumption or blood lactate levels were not modified by repeated plasma donation, whatever the intensity of the donation. One previous study looked at the effects of one single plasma donation on the time to exhaustion, maximum oxygen consumption and markers for anaerobic capacity, that is, blood lactate levels and maximum accumulated oxygen deficit [15]. Although the maximum oxygen consumption was unaffected by plasma donation, time to exhaustion, blood lactate levels and maximum accumulated oxygen deficit were all decreased by 10%–20%. Our results suggest that the negative effects of acute plasma donation on endurance performance are not observed in the longer term when donation is repeated and performance is determined a few days after the last donation in basal conditions. It is important to highlight that, despite the down-regulation of key haematological parameters, endurance performance was not affected. We previously found that a partial dissociation and/or delay may be found between the regulation of haematological parameters, and more particularly those related to the iron status, and endurance performance after repeated blood donation [16, 24]. Given the importance of iron status for exercise performance [25], it cannot be excluded that the 3-month period of investigation was too short to induce detectable down-regulation of endurance and strength performance in the VHF group.

Finally, no serious adverse events were reported, and only a few events (anaemia, haematomas, vasovagal reactions without syncope) were present in the HF and VHF groups, which are classically reported by others after plasma donation [4, 12, 13].

VHF donation affects the IgG levels of donors down to a level that may impact their immune system and may affect haematological parameters. Therefore, countries should invest in building large donor bases to ensure a sustainable plasma supply while avoiding potential negative health effect to their donors.

This study has some limitations. First, only middle-aged men were included in this study and only one new donor was recruited. Therefore, the external validity is limited and the present results cannot be extrapolated to new donors or to female or elderly donor populations. Further studies should investigate whether those results are similar in women and older people.

Second, this randomized controlled trial lasted for 3 months. It cannot be excluded that the severe haematological and biochemical changes measured during this period in the VHF group, although not falling below normal values for most of them, will not affect physiological function and exercise performance in the longer term.

Third, the randomization procedure was sub-optimal since allocation to the HF/VHF group was sometimes in conflict with the availability of the participant. Therefore, the donor was assigned to the first available position on the randomization list that did not conflict with his availability. The impact of this sub-optimal randomization procedure was considered limited.

In conclusion, VHF plasmapheresis may result in a large reduction in ferritin and IgG levels. HF and VHF plasmapheresis may result in little to no difference in other biochemical, haematological, clinical, physiological and exercise-related parameters.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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