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ORIGINAL PAPER

Psychological and hormonal stress response patterns during a blood donation

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Vox Sanguinis

Background and Objectives Donating blood has been associated with increased stress responses, with scarce evidence indicating that levels of psychological and hormonal stress are higher pre-donation than post-donation. We investigated whether a blood donation induces psychological and/or hormonal stress during the course of a blood donation, and whether responses differed between men and women, first-time and experienced donors and donors with high or low non-acute stress.

Materials and Methods In 363 donors, psychological (donation-stress and arousal) and hormonal (cortisol) stress were measured by questionnaire and salivary sample at seven key moments during a routine donation. Non-acute stress was assessed by a questionnaire. Repeated measurement analyses were performed, using the last measurement (leaving the donation center) as reference value.

Results Levels of donation-stress, arousal and cortisol were significantly higher during donation than when leaving the donation center. When compared with men, women reported higher levels of donation-stress and cortisol in the first part of the visit. When compared with first-time donors, experienced donors reported lower levels of donation-stress during the first part of the visit, and higher levels of arousal but less reactivity throughout the visit. When compared to donors high on non-acute stress, donors low on non-acute stress reported lower levels of donation-stress during the first part of the visit, and showed less cortisol reactivity throughout the visit.

Conclusion Donating blood influences psychological and hormonal stress response patterns. The response patterns differ between women and men, first-time and experienced donors and between donors high and low on non-acute stress.

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Introduction

Blood donation has been associated with both positive [1] and negative donation aspects [2–4] for the donor. As people differ in the way they perceive a situation as stressful, the blood donation process may also impact donors differently. Indeed, a number of studies

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summarized by Hoogerwerf *et al.* [5] in a recent review indicate that a routine whole-blood donation is associated with psychological and hormonal stress. We showed that pre-donation levels of psychological stress are reported to be higher than post-donation-stress levels, and pre-donation cortisol levels in first-time donors are reported to be higher than post-donation levels. Taken together, it seems likely that a routine blood donation induces stress responses in healthy blood donors.

Common stress reactions described in the literature include multiple psychological stress reactions, such as increased levels of arousal, anxiety, fear or tension [6, 7], as well as hormonal stress reactions, such as increased cortisol excretion [8]. It is important to note that only moderate overlap between anxiety and stress has been found (at around 0·5 [9, 10]). In a response to acute stress, activation of the hypothalamic–pituitary–adrenal axis results in temporary elevations of circulating cortisol, superimposed on the normal circadian rhythm. Thereby, cortisol reactivity is defined as an increase in at least 2·5 nmol/l above the baseline [11]. A factor that is known to enhance a stress response is unfamiliarity with a situation [12]. In line with this, as the number of previous donations increases, the levels of psychological and hormonal stress have been shown to decrease, as expressed by lower levels of anxiety and cortisol [7, 13–21]. Other factors known to influence psychological or hormonal stress reactions are gender and levels of non-acute stress. Depending on the type of challenge and stress reaction assessed, inconsistent effects are reported for gender [21–24]. Non-acute stress comprises the wide range of daily hassles or minor daily pressures that may lead to the experience of stress. Thereby, higher levels of acute stress when driving a car in heavy or light traffic have been reported in subjects with high levels of non-acute stress [25]. Altogether, a number of factors, including the number of previous donations, gender and levels of non-acute stress, potentially influence a stress response during a donation.

Most studies assessing stress and blood donation have measured stress, but predominantly anxiety only, at two moments: before and after the donation [7, 13, 14, 17, 18, 20, 26]. Although a more detailed analysis of a blood donation procedure has been performed by three other studies, they also included only psychological anxiety measures. Of these studies, Hanson *et al.* assessed anxiety at three moments around the donation, and found anxiety levels were higher during the actual donation than before or after it [21]. Using also three measurement moments, Breckler *et al.* [16] found high levels of anxiety up to needle insertion, and a steep decrease hereafter. Finally, Basler *et al.* [15] assessed anxiety at six moments around a donation, and showed stress levels were highest immediately before venipuncture. This limited body of knowledge makes it difficult to assess the stress response in more detail and to make a systematic comparison between the various moments in a donation procedure. Summarizing, to date, the various key moments in the donation procedure, for example arrival, medical check, needle insertion and uncoupling, and arriving at the donor canteen, have never been systematically assessed using different stress measures.

We set up a study to investigate stress induced by blood donation at multiple key moments, combining psychological and hormonal measurements. We addressed the following research question: does a blood donation induce psychological and/or hormonal stress in whole-blood donors, and are there differences between men and women, first-time and experienced donors and donors with high or low non-acute stress? Based on the literature, we formulated four hypotheses: (1) a blood donation induces a stress response, in which stress peaks around needle insertion; (2) women and men show a different stress response during a blood donation; (3) first-time donors experience more stress than experienced donors; and (4) donors with high levels of non-acute stress exhibit higher levels of acute stress than donors with lower levels of non-acute stress. By combining multiple stress measures and key moments during a donation, we aimed to gain insights into the stress response patterns throughout a whole-blood donation procedure, potentially useful for devising and modifying interventions to minimize negative donation aspects.

Materials and methods

Participants and informed consent

This study is the first part of a larger study investigating donation-induced stress responses and their effect on donor haemostasis (DISTRESS). A random sample of first-time and experienced male and female donors was invited by letter to participate in the DISTRESS study. Three inclusion criteria were formulated: no use of corticosteroids such as asthma medication, as they interfere with the measurement of cortisol in saliva; no use of NSAIDS such as aspirin in the four days prior to donation, as they affect platelet functioning; and no use of beta blockers such as atenolol, as they affect blood pressure. Figure 1 provides a flow chart of the inclusion of the participants.

The DISTRESS study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam and the Ethical Advisory Committee of Sanquin, and conducted in accordance with the principles of the Declaration of Helsinki (64th WMA General Assembly, version October

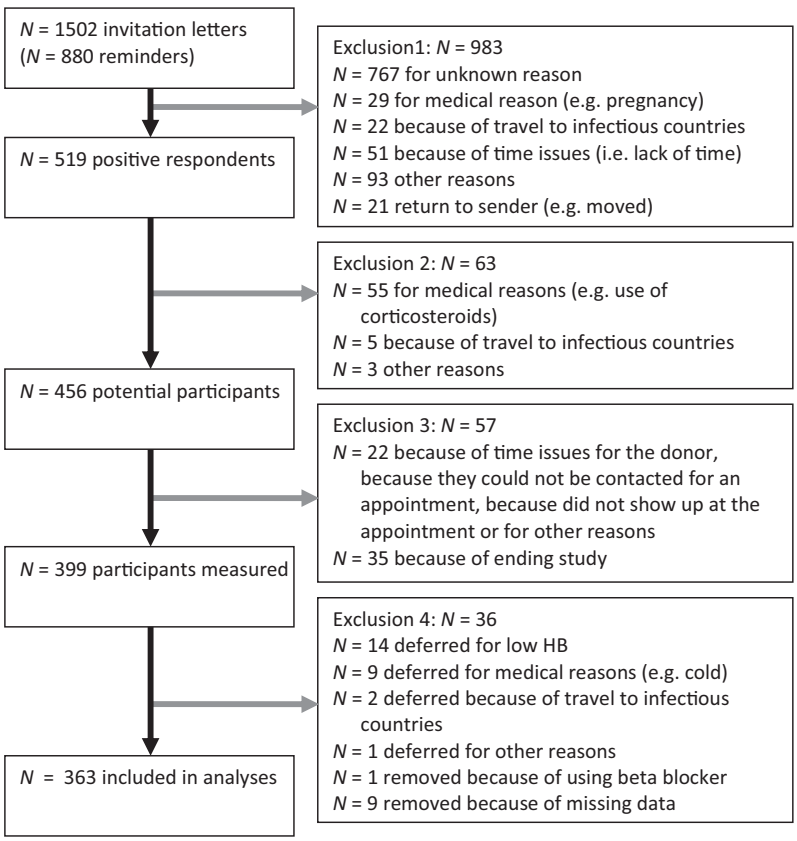

Fig. 1 Flow chart of participant inclusion.

2013, Fortaleza Brazil). Reporting was done according to the Strobe Statement (version 4, October 2007).

Study procedure

On a response card or via e-mail, donors could indicate willingness to participate. If they did not respond to the initial invitation letter, one reminder was sent after four weeks and an attempt was made to contact the donor by phone. Subjects willing to participate were phoned, to arrange a donation. During the pre-donation talk with the researcher, the donor had the opportunity to ask questions about the study, after which informed consent was obtained.

Routine donation procedure

A routine blood donation procedure in the Netherlands starts with the donor reporting at the registration desk. Here, the donor's identity is checked and the donor receives a donation health questionnaire (DHQ). The donor then usually has to wait several minutes before a health screening for eligibility is performed by a donor nurse or physician. This entails measuring capillary haemoglobin level with a photometer (HemoCue Hb 201+, Angelholm, Sweden), measuring heart rate and blood pressure (Omron HEM-907XL, Lake Forest, IL, USA) and evaluating the completed DHQ. If found eligible to donate, the donor is directed to the donation area. Here, the donor must sometimes wait several minutes until a bed or staff member is available. The donor sits and a nurse performs the routine venipuncture, after which blood collection starts. The blood collection process usually takes about 8–13 minutes for whole-blood donors, in semi-recumbent position. The donor is then directed to the donor canteen, where she/he can have a drink and something to eat before leaving the donation center.

Study protocol

After obtaining informed consent, the first visual analog scale (VAS) questionnaire assessing stress and arousal was completed and a non-invasive saliva cortisol sample was taken (measurement one). Next, height and weight were measured. Then, the routine blood donation procedure started with additional measurements (VAS questionnaire, cortisol) on the following key moments: reporting at the registration desk (measurement two), immediately before the screening (measurement three), at needle insertion (measurement four) and immediately after removal of the needle (measurement five). Upon arrival in the donor canteen, the donor was requested to also complete a

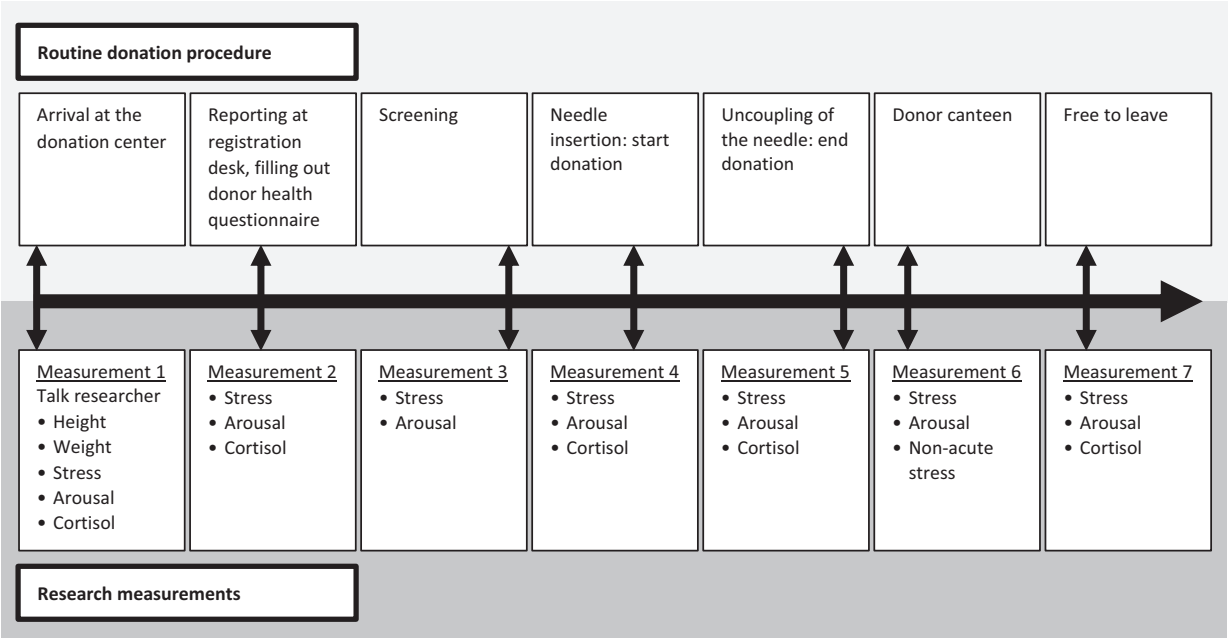

Fig. 2 Set-up of the study procedure. Key moments of a routine donation are shown above the horizontal arrow, whereas additional measurements for the current study are below the horizontal arrow.

questionnaire assessing non-acute stress (measurement six). Hereafter, the donor reported to the researcher (measurement seven). Fig. 2 gives a detailed overview.

Study variables

Psychological stress

Psychological levels of stress were measured during the donation procedure using a hard copy VAS assessing donation-stress and arousal. The scales were a vertical bar of 120 mm, with anchors at 10 and 110 mm labelled 'not stressed/aroused' and 'very stressed/aroused', respectively. Subjects were instructed to answer the two questions (VAS stress: How stressed are you right now? VAS arousal: How aroused are you right now?) by drawing a horizontal line across the vertical line between the two anchors. A definition and sample of the assessed variable was provided. Distance to the lowest anchor was measured in mm, which resulted in a score between 0 (not stressed/aroused) and 100 (very stressed/aroused).

Hormonal stress

Cortisol concentrations (nmol/l) were obtained by taking non-invasive saliva cortisol samples, using Salivettes (Sarstedt, Etten-Leur, The Netherlands). Subjects were asked to gently chew on a Salivette for approximately one minute. Samples were stored at Sanquin at -80°C until sending.

Non-acute stress

To obtain a comprehensive impression of non-acute stress, three forms of non-acute stress were assessed using a digital questionnaire: distress, work-related stress and stress from private life. Donors' distress was assessed using the Distress Screener, a dimension from the 4-DKL [9], which is a reproducible and valid measure. Donors had to rate three statements (During the past week, did you suffer from worry? During the past week, did you suffer from listlessness? and During the past week, did you feel more tense?) on a three options response scale (No (0); Sometimes (1); Regularly or more often (2)). A total score, defined as Distress, was obtained by summing the three items.

Work-related stress and stress from private life were assessed by rating two self-constructed statements (How often have you suffered stress in the past month because of work; How often have you suffered stress in the past month in private life?), on a five options response scale (Never/almost never (0); Sometimes (1); Regularly (2); Often (3); Almost always/Always (4)).

Demographics

Date of birth (to calculate age) and lifetime number of donations were obtained from the blood bank databank (eProgesa, MAK system, France). For weight and height

measurements, subjects were requested to remove shoes, heavy clothing (e.g. jackets) and to empty their pockets. Weight was measured using a digital balance (Seca 888, Hamburg, Germany). Height was measured using a wall-mounted stature meter (Stanley Microtoise 04-116, Besançon, France). Body mass index (BMI) was calculated from height and weight (BMI = weight/height^2).

Analyses

Donors were included in the analyses if they had no missing values for the study variables. Deferred donors (e.g. because of a low haemoglobin level) were excluded from analyses.

Cortisol analysis

Analyses were performed by Technische Universität Dresden, Germany. The first batch (samples from 114 donors) was sent in April 2015. A second batch (samples from the remaining 285 donors) was sent in May 2016. In Dresden, all saliva samples were frozen and stored at −20°C until analysis. After thawing, Salivettes were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity. Salivary concentrations were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). Sample and reagent handling was semi-automated, using a liquid handling robot (Genesis, Tecan, Switzerland), and quality control samples of low, medium and high cortisol concentrations were run on each microtiter plate assayed. The intra- and interassay coefficients for cortisol were both below 8%.

Levels of salivary cortisol start increasing directly after onset of the stressor. Peak response has been reported to vary from 9 min [27], to 21–40 min after onset of the stressor or 0–20 min post-stressor [28], also depending on severity of the stressor [29], or subject reactivity [30]. To facilitate interpretation, we calculated times between consecutive moments and reinterpreted the 'actual' moments of the cortisol samples. Thus, samples obtained on arrival at the donation center provide information from shortly (9–40 min) before arrival. Samples obtained when reporting at the registration desk provide information around arrival (average interval: 13 min). Samples obtained at needle insertion provide information around the screening (average interval: 16 min). Samples obtained at uncoupling of the needle provide information for shortly before needle insertion (average interval: 10 min). And finally, samples obtained when leaving the donation center provide information on sitting in the donor canteen (average interval: 19 min).

Non-acute stress

A combination score was constructed, with donors scoring below or on the median for all three forms of non-acute stress, that is distress, stress from work and stress from private life, assigned to the group with low non-acute stress. Donors scoring above the median for one or more of the three forms of non-acute stress formed the group with high non-acute stress.

Statistical analyses

Psychological and hormonal stress responses were analysed using a repeated measurement model. As post-donation levels of stress have been reported as lower than pre-donation levels of stress [7, 13–18, 20, 21, 26], measurement moment seven was used as reference moment. Difference scores were calculated for the dependent variables (stress/arousal/cortisol [1–6] minus stress/arousal/cortisol [7]) on which analyses were performed. Analyses were executed with the dependent variables as within-subject variables, and, if applicable, group as between-subjects variable (i.e. gender, donation experience, non-acute stress). The effect of time on the difference scores was examined by transforming the scores into linear and quadratic trend contrast scores. The effect of group (gender, donation experience, non-acute stress) was investigated by the interaction effect. Significant effects of group on the linear and quadratic trends across time were investigated further by means of repeated effect tests, to test trend components between groups.

Results

In total, 363 whole-blood donors were included in the analyses (Fig. 1). <5% (n = 9) had to be excluded from analyses because of random missing values, hence imputation was not applied. Donor and donation characteristics are presented in Table 1.

Stress response patterns during a donation

For donation-stress, the analyses (Table 2) revealed a significant effect of time ($F_{5,358}$ = 66·510, P < 0·001). As illustrated by Fig. 3a, levels of donation-stress in the first part of the visit were elevated, peaking at needle insertion and decreased afterwards.

For arousal, results of the analyses (Table 2) showed a significant effect of time ($F_{5,358}$ = 11·429, P < 0·001). Figure 3a shows a predominantly stable score for arousal, slightly elevated at the first part of the visit and increasing slightly at needle insertion.

For cortisol, a log transformation was applied because of skewness towards lower values. Results of the analyses (Table 2) showed a significant effect of time

Table 1 Donor characteristics and outcomes for descriptive variables[a]

		Gender		Donation experience		Non-acute stress	
	Total	Men	Women	First-time	Experienced	Low	High
Number	363	183	180	176	187	140	223
Age (years)	40 ± 16	43 ± 16	37 ± 14	28 ± 9	52 ± 11	44 ± 17	38 ± 14
Previous donations (n)	6 (0–38)	13 (0–53)	0 (0–29)	0 (0–0)	37 (22–61)	25 (0–44)	0 (0–32)
BMI (kg/m^2)	24·6 ± 3·9	24·6 ± 3·2	24·5 ± 4·4	23·9 ± 3·8	25·2 ± 3·8	24·9 ± 3·6	24·3 ± 4·0
Donation-stress at measurement 7[b]	16·2 ± 16·5	16·1 ± 15·2	16·3 ± 17·8	16·5 ± 14·5	15·9 ± 18·3	11·6 ± 13·5	19·1 ± 17·6
Arousal at measurement 7[b]	67·5 ± 25·3	67·3 ± 25·2	67·6 ± 25·5	59·7 ± 26·1	74·7 ± 22·3	74·6 ± 24·4	63·0 ± 24·9
Cortisol at measurement 7 (nmol/l)	5·95 ± 6·81	3·04 ± 7·23	5·85 ± 6·38	7·36 ± 8·87	4·62 ± 3·54	5·83 ± 5·44	6·02 ± 7·55
Distress, past 7 days[c]	1 (0–2)	1 (0–2)	2 (1–3)	2 (1–3)	1 (0–2)	0 (0–1)	2 (2–3)
Work-related stress, past month[d]	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (0–1)	2 (1–3)
Stress private life, past month[d]	1 (1–1)	1 (0–1)	1 (1–2)	1 (1–2)	1 (0–1)	1 (0–1)	1 (1–2)

[a]Presented as mean ± SD or median (25–75 percentile).
[b]0–100 point scale.
[c]0–6 point scale.
[d]0–4 point scale.

Table 2 F and *P*-values for the analyses for donation-stress, arousal, and cortisol

Effect/contrast	Donation-stress	Arousal	Cortisol
Main effect time	$F_{5,358} = 66{\cdot}510$	$F_{5,358} = 11{\cdot}429$	$F_{3,356} = 6{\cdot}660$
	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P < 0{\cdot}001$
Linear effect time	$F_{1,362} = 9{\cdot}021$	$F_{1,362} = 14{\cdot}072$	$F_{1,358} = 19{\cdot}610$
	$P = 0{\cdot}003$	$P < 0{\cdot}001$	$P < 0{\cdot}001$
Quadratic effect time	$F_{1,362} = 196{\cdot}593$	$F_{1,362} = 20{\cdot}093$	$F_{1,358} = 0{\cdot}190$
	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P = 0{\cdot}663$

($F_{3,356} = 6{\cdot}660$, $P < 0{\cdot}001$). Figure 3a shows the untransformed values to ease interpretation: levels of cortisol show an overall decrease during the visit, indicating a decrease in stress.

Subgroup analyses

Table 3 presents results for the analyses of subgroups (gender, donation experience and non-acute stress).

Gender differences

For donation-stress, a significant interaction effect was found for time and gender ($F_{5,357} = 2{\cdot}629$, $P = 0{\cdot}024$). A repeated effect test indicated interaction occurred at needle uncoupling: before this, women scored overall higher than men; after this, no gender differences were apparent (Fig. 3b).

For arousal, no significant interaction effect was found ($F_{5,357} = 0{\cdot}893$, $P = 0{\cdot}486$), indicating that throughout the donation, arousal scores were similar for men and women (Fig. 3b).

For cortisol, a significant interaction effect was found between time and gender ($F_{3,355} = 2{\cdot}821$, $P = 0{\cdot}039$). A repeated effect test indicated interaction occurred at the screening: before this, women scored overall higher than men; after this, no gender differences were apparent (Fig. 3b).

Donation experience

For donation-stress, a significant interaction effect was found for time and donation experience ($F_{5,357} = 5{\cdot}278$, $P < 0{\cdot}001$). A repeated effect test indicated that significant differences occurred at needle insertion, at needle uncoupling and at the donor canteen. As Fig. 3c shows, levels of donation-stress were elevated in both groups in the first part of the visit. Compared with experienced donors, first-timers start with a higher level of donation-stress, and showed a larger and higher peak during needle insertion. Scores are comparable when leaving the donation center.

For arousal, a significant interaction effect was found for time and donation experience ($F_{5,357} = 5{\cdot}278$,

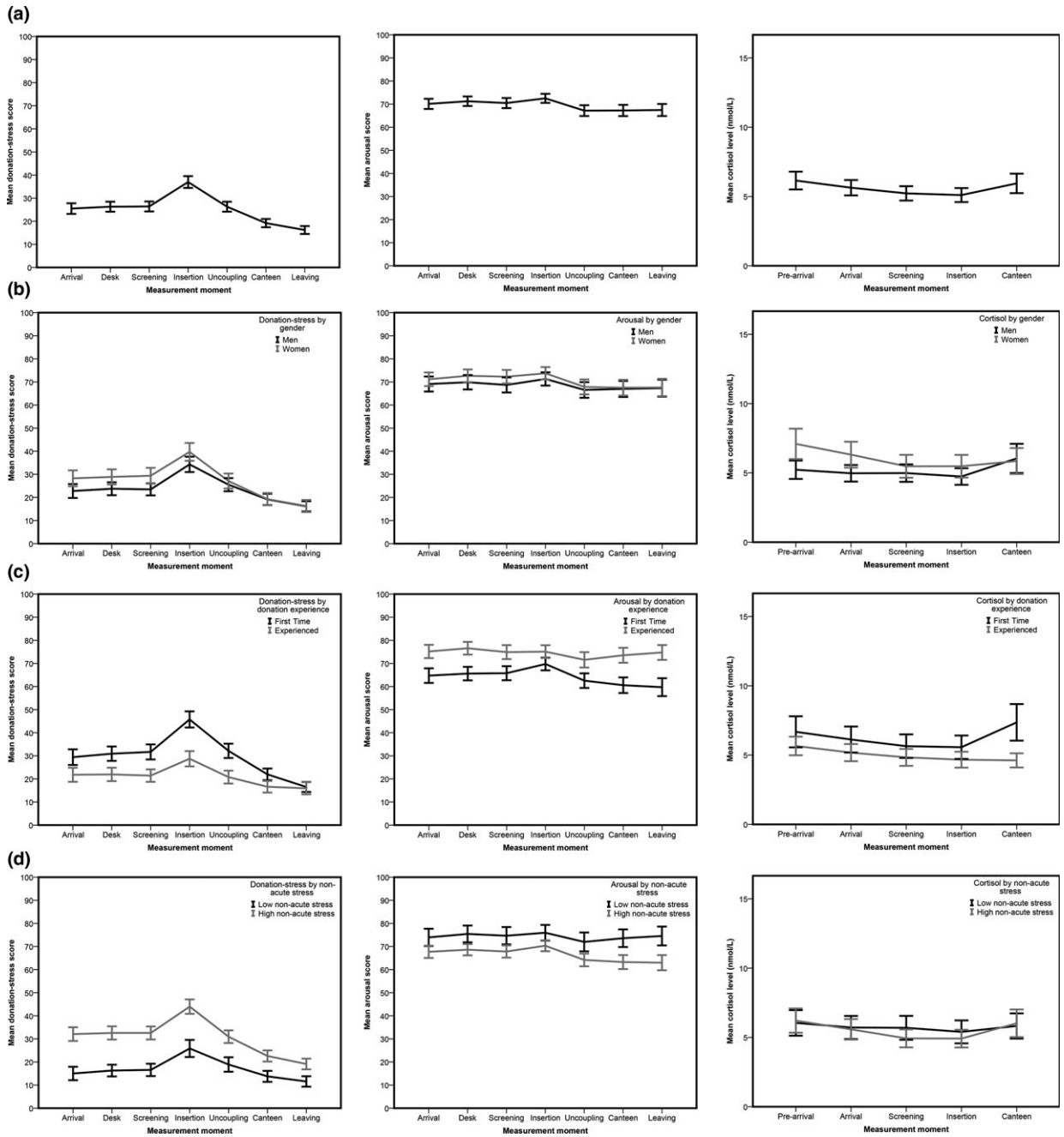

Fig. 3 Means of the different stress scores (donation-stress, arousal, and cortisol), including 95% CI error bars at key moments during a whole-blood donation, for total group (a), gender (b), donation experience (c) and non-acute stress (d).

$P < 0{\cdot}001$). A repeated effect test indicated interaction occurred at the screening, at needle insertion, at needle uncoupling and at the donor canteen. Figure 3c shows that experienced donors scored higher and less reactivity than first-timers at all these moments.

For cortisol, no significant interaction effect was found for time and donation experience ($F_{3,355} = 1{\cdot}354$, $P = 0{\cdot}257$), indicating that throughout the donation, cortisol levels were similar for first-time and experienced donors (Fig. 3c).

Non-acute stress

After constructing the two groups for non-acute stress, the group low in non-acute stress comprised 140 donors. The group high in non-acute stress comprised 223 donors.

Table 3 F and *P*-values for the analyses for donation-stress, arousal and cortisol, with repeated measures and between-subjects variable group

	Effect/contrast	Donation-stress	Arousal	Cortisol
Gender*time	Interaction effect	$F_{5,357} = 2{\cdot}629$	$F_{5,357} = 0{\cdot}893$	$F_{3,355} = 2{\cdot}821$
		$P = 0{\cdot}024$	$p = 0{\cdot}486$	$p = 0{\cdot}039$
	Linear effect	$F_{1,361} = 7{\cdot}615$	$F_{1,361} = 1{\cdot}020$	$F_{1,357} = 2{\cdot}848$
		$p = 0{\cdot}006$	$p = 0{\cdot}313$	$p = 0{\cdot}092$
	Quadratic effect	$F_{1,361} = 4{\cdot}144$	$F_{1,361} = 2{\cdot}116$	$F_{1,357} = 4{\cdot}018$
		$p = 0{\cdot}043$	$p = 0{\cdot}147$	$p = 0{\cdot}046$
	Interaction point	Moment 4 vs. 5		Moment 2 vs. 4
Donation experience*time	Interaction effect	$F_{5,357} = 9{\cdot}132$	$F_{5,357} = 5{\cdot}278$	$F_{3,355} = 1{\cdot}354$
		$p < 0{\cdot}001$	$p < 0{\cdot}000$	$p = 0{\cdot}257$
	Linear effect	$F_{1,361} = 0{\cdot}035$	$F_{1,361} = 0{\cdot}049$	$F_{1,357} = 1{\cdot}243$
		$p = 0{\cdot}853$	$p = 0{\cdot}824$	$p = 0{\cdot}266$
	Quadratic effect	$F_{1,361} = 32{\cdot}538$	$F_{1,361} = 14{\cdot}440$	$F_{1,357} = 2{\cdot}767$
		$p < 0{\cdot}001$	$p < 0{\cdot}001$	$p = 0{\cdot}097$
	Interaction point	Moment 3 vs. 4, 4 vs. 5, 5 vs. 6	Moment 2 vs. 3, 3 vs. 4, 4 vs. 5, 5 vs. 6	
Non-acute stress*time	Interaction effect	$F_{5,357} = 5{\cdot}628$	$F_{5,357} = 1{\cdot}425$	$F_{3,355} = 3{\cdot}093$
		$p < 0{\cdot}001$	$p = 0{\cdot}214$	$p = 0{\cdot}027$
	Linear effect	$F_{1,361} = 13{\cdot}602$	$F_{1,361} = 2{\cdot}744$	$F_{1,357} = 3{\cdot}311$
		$p < 0{\cdot}001$	$p = 0{\cdot}099$	$p = 0{\cdot}070$
	Quadratic effect	$F_{1,361} = 8{\cdot}705$	$F_{1,361} = 2{\cdot}950$	$F_{1,357} = 5{\cdot}364$
		$p = 0{\cdot}003$	$p = 0{\cdot}087$	$p = 0{\cdot}021$
	Interaction point	Moment 4 vs. 5, 5 vs. 6		Moment 2 vs. 4

For donation-stress, a significant interaction effect was found for time and non-acute stress ($F_{5,357} = 5{\cdot}628$, $P < 0{\cdot}001$). A repeated effect test indicated interaction occurred at needle uncoupling and in the donor canteen. As can be seen in Fig. 3d, donors with high non-acute stress not only showed a higher level of donation-stress, but also had higher levels of donation-stress in the first part of the visit, and a steeper decline after needle insertion.

For arousal, no significant interaction effect was found for time and non-acute stress ($F_{5,357} = 1{\cdot}425$, $P = 0{\cdot}214$), indicating that throughout the donation, arousal levels of first-time and experienced donors were similar (Fig. 3d).

For cortisol, a significant interaction effect was found for time and non-acute stress ($F_{3,355} = 3{\cdot}093$, $P = 0{\cdot}027$). A repeated effect test indicated interaction occurred at the screening: donors low in non-acute stress showed less cortisol reactivity than donors higher in non-acute stress (Fig. 3d).

Discussion

Our findings provide detailed insight into psychological and hormonal stress responses during a blood donation procedure. We found a clear donation-induced response in donation-stress, which varied between the different groups of donors: women, first-time donors and donors high on non-acute stress reported higher levels of donation-stress when compared to men, experienced donors and donors low on non-acute stress, respectively. Moreover, a high and constant level of arousal was observed, indicating that donors were aroused throughout the visit. Levels of cortisol decreased during a donation when analysing the total group, and group differences were found, indicating lower levels of cortisol up to the screening for men compared to women, and a lower cortisol response for donors low compared to high on non-acute stress.

With respect to hypothesis (1), we found lower levels of donation-stress at the end of a donation. These results largely agree with previous findings, assessing predominantly anxiety [7, 13, 14, 18, 20, 26]. In line with our hypothesis two out of three articles assessing multiple moments [15, 21], we found donation-stress peaked during needle insertion. At first sight, anxiety and donation-stress might be expected to coincide considerably, but research suggests only moderate overlap, with stress and anxiety correlating at around 0·55 [9, 10]. Donation-stress therefore covers a more broad range, also including nervousness and tension [6]. Our results for arousal indicate a relatively high arousal level and a small yet significant decrease during donation, also reported by Ferguson *et al.* [7] Our results for cortisol partly conform with those reported by others, whereas cortisol decreased in all our donors towards the end of the donation procedure, Bellitti *et al.* [17] found that during a donation, serum cortisol decreased in first-time donors but not in experienced donors. The disparity may result from differences in cortisol sampling: Bellitti *et al.* used a fixed sampling

time after donation, whereas we sampled cortisol at fixed key moments during the donation procedure.

Regarding hypothesis (2), we found that compared to men, women expressed higher levels of donation-stress and cortisol in the first part of the donation. There is no literature investigating gender differences in stress responses in a blood donation setting, but in a non-donation setting, salivary cortisol levels indicated women were more physiologically reactive to social rejection challenges, whereas men reacted more to achievement challenges [22]. It could be argued that social acceptance, the feeling of being accepted, played a role in our finding that women were more reactive in the first part of the visit. This part of the donation procedure primarily focuses on eligibility to donate, thus potentially perceived as being accepted or not. In contrasts, men had increased cortisol levels during the last part of the donation visit, which is when the focus is on being able to successfully complete an actual donation, which might be perceived as an achievement.

Our results concerning hypothesis (3) indicate that compared to experienced donors, first-timers expressed higher levels of donation-stress in the first part of the donation. This is partly in line with previous results [7, 13, 14, 18, 20, 26]. Although a significant group effect was found for arousal (experienced donors showing higher arousal levels), both groups scored relatively high, indicating that both were clearly aroused throughout the procedure. Our finding that cortisol level was not affected by donation familiarity contrasts with the findings of Bellitti *et al.* [17], who found higher levels of pre-donation serum cortisol at the first donation than at the fourth donation. However, they collected serum cortisol by an additional venous blood sample instead of non-invasive saliva samples, so their donors experienced two additional venous blood samples on top of the regular phlebotomy procedure. It is conceivable that these two additional samples caused additional stress and were therefore (at least partly) responsible for the raised cortisol levels in first-time donors.

Finally, regarding hypothesis (4), we found higher scores in donation-stress for donors with high non-acute stress than for donors with low non-acute stress, which is in line with the literature [25]. Despite the significant effect for cortisol, differences on group level were below the earlier used threshold of 2·5 nmol/l [11] and are therefore considered as changes that are not relevant.

Limitations

Salivary cortisol has a delayed response, which hampers interpretation. In our design, to minimize stress caused by the study, we aimed for minimal invasiveness, both in types of measurements and interference in the routine donation procedure. We therefore assessed cortisol at the key moments, together with the questionnaire on donation-stress and arousal. The interval between consecutive measurement moments was variable between donors, mainly due to differences in waiting or relaxing time. Although we thus might have missed peaks in the cortisol response, this seems unlikely, as stable cortisol levels with small confidence intervals were found across groups and measurement moments.

The level of non-acute stress may impact upon the level of stress experienced during a donation. Non-acute stress and the number of donations were both assessed using a continuous scale and transposed to dichotomous variables in our analyses. In future, it might be interesting to gain insight into the number of donations after which novice donors learn to cope with the donation procedure and their stress level approaches that of experienced donors.

Finally, the study itself might have had an impact on the results. For instance, the sampling of saliva using Salivettes or the sampling of psychological stress measures might be considered as unpleasant. However, the multiple evaluation moments of donation-stress and arousal and cortisol sampling proved useful as we showed a non-linear relationship between the successive measurement moments. Measuring stress, for example anxiety, only before and after the phlebotomy therefore seriously underestimates the donation-stress experienced by the donor, which might be of use when evaluating the effects of anxiety on return behaviour or vasovagal responses.

To conclude, we found a donation-induced effect on the level of donation-stress, which was apparent, although to some extent different, among different donor groups. Although absolute levels of donation-stress were low, we found a clear peak during needle insertion. Moreover, we found distinct differences between groups, most explicitly between first-time and experienced donors. Despite some small group differences, all donors indicated a high level of arousal. Differences in the levels of cortisol were small and therefore non-relevant. To our knowledge, ours is the first study to systematically evaluate donation-induced stress at multiple key moments in the donation procedure. We have also provided new insights into the course of the stress reaction itself. Although phlebotomy remains necessary, blood bank personnel may benefit from the scientific evidence that different donors respond differently to the various components of a blood donation procedure. However, our research needs further replication to gain more insight into the relevance of the stress response, for example concerning the

need for stress reduction or the effects of increased stress on return behaviour and into the group differences found, for example by evaluating interaction effects between gender and donation experience. Moreover, also physiological stress responses might be involved, as well as stress-induced effects on coagulation.

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