Associations of environmental characteristics and lifestyle behaviours with donor blood parameters

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- CHAPTER EIGHT -

General discussion

The project described in this thesis aimed to study associations of lifestyle behaviours, and potential built-environmental determinants or correlates of such behaviours, with donor blood parameters. This was further specified in the following three research objectives:

◊ Examine to what extent and via which lifestyle behaviours built-environmental characteristics are associated with blood lipid levels

◊ Examine to what extent lifestyle behaviours of donors are associated with haemolysis and haemoglobin levels and study the mediating role of blood lipids and ferritin

◊ Examine to what extent iron status is associated with physical activity and physical capacity.

This chapter starts with summarising the main findings of the conducted research. Subsequently the methodological considerations of and reflections on the findings are presented. Thereafter recommendations for research and implications for practice, and overall conclusions are provided.

Main findings

In **chapter 2**, we describe a systematic review with meta-analyses which showed that residents in urban areas have less favourable blood lipid profiles (i.e., higher total cholesterol, higher Low Density Lipoprotein (LDL) and higher triglyceride levels), compared to residents in rural areas. No differences were identified in High Density Lipoprotein (HDL). We further describe in **chapter 2** that the majority of these studies were conducted in low- and middle income countries and that the eligible included studies did not enable us to study mediation of lifestyle behaviours. This led us to further investigate this association in a large geographically well-distributed cohort in the Netherlands, i.e. a high income country. In **chapter 3** we describe this donor cohort -Donor InSight (DIS)-, which we also used to address our second research objective, in detail. We showed that DIS respondents were generally representative of the Dutch donor population. We expanded the existing knowledge on urban and rural differences in the Netherlands using population density because this enhances comparability with results of studies done in other high income countries. No meaningful and statistically significant differences were found in blood lipid levels across different population densities, neither was evidence found of mediation by objectively measured or self-reported physical activity or sedentary behaviour.

In **chapters 5** and **6**, we examined associations of lifestyle behaviours of donors with haemolysis and haemoglobin levels in DIS; including mediating roles of blood lipid and ferritin levels therein. We found that objectively measured

physical activity and sedentary behaviour, and self-reported consumption of nuts, fish, meat and eggs were not associated with haemolysis levels in miniature red cell concentrates. Neither were there any associations between these indicators of lifestyle behaviours and LDL and triglyceride levels. We did demonstrate in **chapter 5** that blood lipids were positively associated with haemolysis. **Chapter 6** presents the results of the assessment of associations of lifestyle behaviours and haemoglobin levels and trajectories and the mediating role of ferritin levels. This study demonstrated that the consumption of haem iron was positively associated with haemoglobin levels, while the consumption of non-haem iron was negatively associated. Adjusting for foods preventing the absorption of non-haem iron largely diminished the negative association. The associations of haem and non-haem iron consumption with haemoglobin were both mediated by ferritin levels. This indicates that the association between dietary iron intake and haemoglobin can be explained by ferritin levels to a large extent.

In a series of studies described in **chapter 7** we consistently demonstrated that a normal iron status was not associated with physical activity and physical capacity in the general population. The first study presents the results from the INTERVAL trial, a large randomised controlled trial in which data from donors allocated to various inter-donation intervals were analysed. We found no evidence of differences between any of the inter-donation interval groups. Neither did we find any indications that some groups of donors (classified according to e.g. age, initial haemoglobin and ferritin levels, use of iron supplementation) were more prone to change their physical activity pattern. Similar null findings were observed in the second study of genetically predicted haemoglobin or ferritin and device-measured physical activity using data from individuals of the general population. The third study consisted of an umbrella systematic review with meta-analyses on iron status and physical capacity outcomes. This review showed that changes in iron status through supplementation only beneficially influenced physical capacity outcomes in anaemic patient. No consistent beneficial effects of iron supplementation on physical capacity were found in iron deficient non-anaemic populations and (recreationally trained) athletes. We concluded that medium-to-long term lower iron status (i.e. lower ferritin levels, but still adequate haemoglobin levels) do not translate into differences in habitual physical activity levels and sedentary behaviour.

Methodological considerations and reflection on findings

We applied a wide range of study designs, various data sources and statistical analyses methods in studying associations between the built environment, lifestyle behaviours and blood parameters. All studies commenced with

hypotheses and were based on (biological) conceptual frameworks. We made extensive research plans including statistical analyses plans which were adhered to during the execution of the studies. Regardless of whether or not outcomes were statistically significant we aimed at publishing all studies. Despite our best efforts, we are aware that the studies conducted have their limitations. These and their potential effect on our results are discussed below. In light of the methodological consideration a reflection on the findings is also presented.

Study designs
Data from the second measurement round of the observational cohort study Donor InSight (DIS) was used for cross-sectional analyses in **chapters 3 – 5**. Measurements of both exposure and outcome at the same time were analysed, which inhibits causal inference. Besides restrictions on causal inference due to the cross-sectional design, reverse causation could have played a role. Only associations for which we could *a priori* establish a plausible biological mechanism were statistically tested. In the case of iron and physical activity it is plausible that the relation is bidirectional and dynamic. Perhaps increased physical activity levels led to higher haemoglobin levels (**chapter 6**), but, conversely, haemoglobin levels maybe were the inhibiting or facilitating factor resulting in differences in physical activity levels (**chapter 7**). For example, individuals with low haemoglobin levels might already face challenges such as shortness of breath whilst climbing stairs, making engagement in more strenuous physical activity unlikely. On the other hand, higher RBC production itself likely does not influence individuals to become more physically active, but higher haemoglobin levels could actually have enabled individuals to become more physically active.

A major issue of studies examining effects of blood donation on donor health outcomes using routinely collected data is that there may be unrelated reasons why some donors donate more than others, and are over-represented in the data[1]. A person experiencing less negative effects of donation such as tiredness or fatigue after a donation is more likely to return for next donations than someone who experienced more negative effects[2]. Apart from self-selection of donors, selection procedures of blood services also contribute to selection bias[3]. These selection effects could lead to false conclusions that donating more often is not associated with negative health outcomes. In this context the large randomized controlled trial (RCT), the 'INTERVAL' trial provides a unique and internally valid design to investigate effects of blood donation intensity on physical activity (**chapter 7**). However, although RCTs are less prone to confounding, if donors allocated to a specific interval were more likely to drop out, this would still have resulted in systematic differences between groups.

We investigated attrition and found that drop out was not associated with donation intensity. However, it seemed that attrition was somewhat related to donor careers or donation experience, with potentially more 'loyal' donors to continue the trial, which warrants caution when generalising findings.

Another study design avoiding reverse causation is Mendelian Randomization (MR), which we used in **chapter 7**. This design uses the principle that genetic variation is randomly assigned at meiosis and as such is not influenced by other characteristics, thereby further reducing risks of (unmeasured) confounding and reverse causation[4-6]. As the findings of the MR analyses corroborated our findings in a more general population, the previously mentioned selection bias due to the attrition and donor (self-) selection is likely to have been limited.

Both systematic reviews with meta-analyses included in this thesis (**chapters 2 and 7**) used predefined study protocols registered before commencement of the actual search for eligible studies (PROSPERO registration: CRD42016043226 and CRD42019123885). The protocols defined clear inclusion criteria and research question(s), which provides transparency and may help to limit heterogeneity of included studies. To prevent reviewer bias, screening of eligible studies was conducted by two reviewers independently which was also done when assessing the risk of bias of the included original studies (**chapter 2**) and the included systematic reviews (**chapter 7**). Like all (umbrella) reviews, ours depended on the quality (and risk of bias) of the eligible studies included. Risk of bias assessment helps interpreting findings and potentially explains identified differences. Indeed, we observed that although the effect estimates of studies rated as weak were of similar magnitude and direction as those with a higher rating, the uncertainty was generally larger. This implied that the confidence intervals were larger compared to studies rated as having moderate or high quality in **chapter 2**.

Study populations

Both DIS and the INTERVAL trial consist of donor populations and were reasonably representative of their respective original donor populations[7,8]. The previously mentioned donor selection bias did not likely pose problems because –with the exception of **chapters 4** and **7**– we did not aim to generalize our findings to other populations than donors[1]. For instance, haemolysis in red cell concentrates is a typical blood bank practise issue (**chapter 5**), blood from people of the general population is not stored in a bag, only blood of donors is. The fact that donors are a relatively healthy sub-population is therefore not an issue. However, the limited variation in blood lipid levels and generally rather desirable levels of blood parameters (that is, low total

cholesterol, low LDL cholesterol and low triglyceride levels) are probably a result of the selection of relatively healthy donors. This made it difficult to identify differences meaningful to public health, and one must be cautious as the generalizability of the observed findings to the general population may be limited.

Measurements

Blood parameters
Blood lipid levels in DIS-III were measured in non-fasting blood samples, and LDL levels were calculated using the Friedewald formula rather than via direct measurements.[9] In particular triglyceride levels can be overestimated using non-fasting samples[10]. The Friedewald formula contains the triglyceride level as parameter, which in turn may influence LDL levels as well[10-12]. This implies that, although the measured LDL and triglyceride levels were generally within the normal range, the levels used in our analysis in **chapters 4 - 6** may still be an overestimation of fasting levels. However, it would be unethical to ask donors who come to donate 500ml of blood to arrive in a fasting state.

To study haemolysis in red cell concentrates (RCC), a model reflecting storage of RCC under routine blood service conditions was imperative. Haemolysis levels estimated by our model used in **chapter 4** were high, probably because the storage conditions in the miniature RCCs were less favourable than those of standard RCCs. Although post-hoc analyses indicated that haemolysis levels were systematically overestimated, the method was shown to be valid. The REDS-III study used an alternative approach and designed a special 'transfer bag' in collaboration with the manufacturer of their routinely used blood bag which had the same properties as their standard blood bag[13]. However, this method requires considerably more funds and time to develop and as such was less feasible.

As both the prevention of allowing donors to donate having inadequate haemoglobin levels to donate (false negative) and unnecessarily deferring donors with acceptable haemoglobin levels (false positive) is undesirable, accurate measurement of haemoglobin is important for blood services. Several methods are used in routine practice, including non-invasive techniques which use spectroscopy and invasive methods which either use capillary or a venous blood samples[14]. Non-invasive methods are preferred by donors, but invasive methods yield better sensitivity results[15]. To measure haemoglobin capillary blood samples are used with a copper sulphate test by the NHSBT, and with a photometer by Sanquin. The gold standard in haematology research is the use of haematology analysers which were used both in DIS-III and the INTERVAL trial[7,8,16]. Therefore, measurement error

is expected to be minimal. DIS-III ferritin levels were measured using the Architect Ci8200, Abbott Laboratories, Illinois, U.S.A) and were validated by the National Sanquin Screening laboratory[8].

Lifestyle behaviours

Moderate-to-vigorous physical activity (MVPA) and sedentary behaviour were assessed using self-reported questionnaires and accelerometers (**chapters 3-7**). Questionnaires are known to be prone to social desirability and recall bias leading to biased estimates[17]. Both under- and overestimation may occur. Dowd et al. concluded in their review on clinimetrics of physical activity measurement measures that, although objective measures (including accelerometers) showed less variability than recall based measures, considerable levels of variability were still discernable[18]. Nonetheless, researchers are recommended to include objective measurement methods[18,19]. While accelerometers objectively measure acceleration, data cleaning and cut-off choices can have a great influence.

Based on recent literature a cut-off point of ≥125 milli-gravity (m*g*) was used for MVPA in **chapter 7**[20,21]. The other commonly used cut-off point in research is ≥100 m*g*, comparing time engaged in MVPA with ≥125 m*g* is evidently higher[22]. This is particularly important for surveillance data and comparing effectiveness of physical activity levels between trials. Levels of physical activity of English donors were higher compared to other population-based studies in the United Kingdom using accelerometers[20,23]. Studies differentiating between health status showed that healthy individuals generally have higher levels of MVPA, although these levels were closer to the levels found in the INTERVAL trial, MVPA of donors was still higher[20,23]. MVPA levels of Dutch donors (**chapter 4**) were similar to levels reported in a Dutch and Belgium urban population[24]. Loyen et al., reported population levels of physical activity in Europeans, showing a wide range of mean MVPA levels per week from 45 to 960 minutes per week, most of these were assessed with self-reported questionnaires[25]. Since we reported accelerometer derived geometric mean levels in **chapter 7**, direct comparison is not possible. However, a rough estimation suggests that one third of the studies reported higher levels of MVPA. To illustrate the potential influence of various cut-off points on both the reported time engaged in levels of physical activity and thus concurrent validity of accelerometers and self-reported questionnaires, we elaborated an example using the findings of **chapter 7**. Time spent in MVPA of women allocated to the 12-week donation-interval, using the most liberal cut-off point results in 115 minutes MVPA per day while applying the 150 m*g* cut-off point reduces this by more than half. Using the first cut-off point results in underestimation by a questionnaire in studying

agreement between measurements, while using the more stringent cut-off point with the same questionnaire data leads to overestimation. Bland and Altman analyses to study the agreement between the measurements (using 125 mg as the standard) in **chapter 7** showed proportional bias, meaning that the mean bias increased when the average of both measurements increased. This, however, is reported in literature more often[26-28]. In **chapters 4, 6** and **7** we used both self-reported and accelerometer derived physical activity variables, and regardless of the measurement method, our findings were of a similar nature enhancing confidence in our results.

We did not find the more active donors to have more desirable blood lipid levels (chapter 4). Dose response effects could potentially explain our findings. Beneficial health effects of physical activity are well-established, the most well-known WHO guideline recommends 150 minutes of MVPA per week29. However, physical activity and health benefits (including more preferable blood lipid levels) show a dose-response relation30,31. With the greatest relative health benefits increase in inactive individuals becoming more active; attenuation of the health effects at higher physical activity levels; and detrimental effects with extreme exercise, a graphical representation is shown in Figure 130-32. Although health benefits do not become apparent after reaching a certain threshold, a systematic review provided quantification on physical activity levels and risk reductions of cardiovascular diseases[31]. Individuals engaging in 150 minutes of MVPA had a 20% lower risk compared to inactive individuals, and those spending 300 minutes per week in MVPA had 20% lower risk[31]. We demonstrated that donors were physically active (**chapter 4** and **7**). A majority of 70% of the DIS-III donors adhered to recommended 150 minutes of MVPA per week (**chapter 4**). It could be argued that for most donors the incremental effect of an additional 10 minutes of physical activity on blood lipid levels was limited.

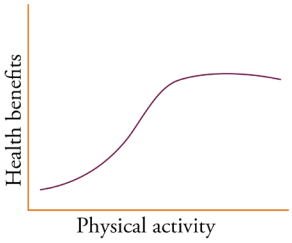

Figure 1: Health benefits of physical activity.

DIS-III was set up to investigate associations of lifestyle behaviours and iron parameters, and the most appropriate questionnaires were chosen for this purpose. The advantage of using a food frequency questionnaire (FFQ) specifically designed to estimate dietary iron intake enabled us to distinguish between haem and non-haem iron intake. Thus far, this differentiation was lacking in donor research, but could clarify the discrepancy (**chapter 5**) with earlier studies[33-35]. We identified contrasting associations, positive for haem iron intake and unexpectedly a negative association for non-haem iron intake. Although haem iron has better bioavailability than non-haem iron,

actual absorption also depends on iron status and the intake of other dietary items[36]. For example, tea and grains (containing polyphenols and phytates) inhibit dietary absorption of non-haem iron[36]. Post-hoc sensitivity analyses revealed that the unexpected negative association of non-haem iron intake and haemoglobin levels attenuated when adjustments were made for phytate-rich and polyphenol-rich food items. More precise measurement informed us that haem iron does play a role for haemoglobin levels in the context of blood donation. Moreover, our study also highlighted the importance of assessing inhibitors and enhancers of dietary iron absorption.

The main disadvantage of this particular FFQ with regard to the current thesis was the inferior estimation of dietary intake of (un)saturated fat, cholesterol and omega-3 fatty acids. The FFQ included food items containing these fatty components, and as such we used these food items in our analyses. Given that the FFQ was not designed to estimate these particular dietary intakes, findings should be interpreted with caution.

Environmental determinants of lifestyle behaviours

The notion that place is relevant for health has been acknowledged and studied by various disciplines, including epidemiology, geography and sociology[37]. Selecting exposures that can capture hypothesized underlying associations is complicated, yet very important[37,38]. Socio-ecological models and complex adaptive system maps imply interactions within and across various layers of determinants, and studying one or just a few aspects in isolation can fail identifying associations[39,40]. Our review on built-environmental characteristics and blood lipid levels (**chapter 2**) showed that the distinction between urban and rural areas of residence was the most common operationalization of residential exposure in literature related to blood lipids in low- and middle-income countries. However, the classification of urban and rural was, ambiguous in the majority of the studies which corroborates with the findings of Allender et al[41]. As the lack of clear definitions makes the use of this rather large spatial scale difficult for comparison, we chose a different operationalisation in **chapter 4**. We could not replicate the findings, which may be explained by the fact that the contrast between more densely populated areas in the Netherlands and less populated areas is too small in terms of environmental exposures.

Statistical analyses

Relevant confounders were tested (**chapter 5 and 6**) or incorporated into the model based on theory (**chapter 4**). However, we may not have included all relevant confounders, or confounders could have been estimated more accurately. For example, it might be more relevant to adjust for

cumulative smoking (pack years) rather than current smoking status as was done in DIS. If a hypothesized confounder is actually part of the causal pathway between the determinant and outcome, adjustment leads to over-adjustment. In such cases, the incorrectly identified confounder is in fact a potential mediator. As we hypothesised some variables to be mediators rather than confounders we used several approaches to assess mediation, including the classical causal steps approach (**chapter 5**) and formal mediation analyses (**chapter 4** and **6**)[42,43]. The merit of mediation analysis is that it provides insights whether for example dietary intake iron and haemoglobin levels are associated through ferritin levels and as such sheds light on biological mechanisms (**chapter 6**). It should however be noted that also with mediation analyses only a limited number of pathways can be studied while in reality it is likely there is a multitude of interconnected pathways.

Despite considerable efforts to minimize missing data, missing data should be acknowledged. As previously discussed, missing data could be due to self-selection and thus attrition which can lead to biased results, in particular with the medium-term follow-up of four years (**chapter 7**). We used an intention-to-treat strategy, meaning that all donors were analysed according to their allocated donation-interval at randomization[44,45]. This implies that donors assigned to the 8-week donation interval, but who in practice donated every 12-weeks, were nonetheless analysed as part of the 8-week interval group. Analysing per protocol, which would entail only analysing those who adhered to their allocated donation-interval while there may very well be reasons related to the donation-interval that are the cause of non-compliance which could lead to incorrect conclusions[45]. Missing data may pose problems also in cross-sectional studies. When using linear regression missing data on any of the included variables leads to the exclusion of individuals, this could lead to a substantial loss of individuals and thus also of statistical power[46]. Therefore, we used multiple imputation in **chapter 4** and **5**[46]. The sensitivity analyses using only complete cases in **chapter 4** showed similar results as the main analyses.

Future research and implications for practice

The work presented in this thesis highlights several leads worthy of further investigation. It also points out opportunities to the research community to collaborate on consensus on variable definitions and measurement methods to improve comparability and pooling of data. Data harmonisation can enhance generalizability of findings. Furthermore, combining data could contribute to investigating research questions for which individual studies are statistically underpowered.

With respect to both physical activity and population density, gaining insight in potential dose-response effects and in threshold effects is recommended. As we found donors to be rather active in terms of physical activity, it would be relevant to further study associations of relevance from a public health perspective, and threshold effect in non-donors. Although we did not find any associations of population density and blood lipid levels, it is evident that more research is required in high-income countries having a more diverse population density in order to shed light on whether or not a certain threshold effect exists. The use of more sophisticated measurements and a combination of environmental characteristics is suggested to better understand the pathways via which the environment influences health outcome. Another notion is the need for consensus on standardization of commonly used measures. This would be desirable with regard to the urban vs. rural distinction, but perhaps even more so for accelerometer-derived physical activity levels. These accelerometer-derived measurements are generally regarded as objective, yet the choices made concerning cut-off points can greatly muddle associations, and limit comparability and pooling across different studies.

Based on the results presented in this thesis we do not recommend blood services to take residential density or the studied lifestyle behaviours of donors into account when recruiting new donors or selecting donors for donation. We did show LDL cholesterol and triglyceride levels to be positively associated with haemolysis levels, replicating laboratory studies and demonstrating this relation in routine practise in a large sample (**chapter 4**). While these blood lipids were positively associated, at least at Sanquin, haemolysis levels do not pose quality control issues. Moreover, given the need and endeavours undertaken by Sanquin to recruit and retain donors, implementing additional selection criteria are – at this point – not recommended. Assessing blood lipid levels could however be considered as a retention strategy; research in Germany has shown beneficial effects of donor health checks on donor return behaviour[47]. As donors with higher haem iron intake were found to have higher haemoglobin levels, it could be interesting to more specifically inform donors about the distinction between haem and non-haem iron. The effects might be small, as it is known that changing dietary behaviour is challenging. Also given the voluntary nature of blood donation, donors should not feel obliged to change their dietary pattern. It is known that donors are a healthy sub-population, and we showed that this also translates into high levels of habitual physical activity (**chapter 7**). Moreover, the knowledge that regular blood donation does not seem to affect physical activity levels or sedentary behaviour is reassuring for both blood services and donors.

Conclusion

This thesis reports on associations of environmental characteristics and lifestyle behaviours with donor blood parameters. In low- and middle-income countries we found that blood lipid levels were less favourable in urban than in rural areas. However, these findings were not replicated in blood donors in the Netherlands, a high-income country. Neither were there indications that lifestyle behaviours mediate associations between population density and blood lipid levels. Our investigations into lifestyle behaviours and blood parameters revealed that donors with a higher haem iron intake had greater iron stores and higher haemoglobin levels. We did not identify evidence of physical activity, sedentary behaviour, or dietary fat intake being related to haemolysis levels in red cell concentrates. Our results did, however, confirm associations between blood lipids and haemolysis levels. Furthermore, this thesis showed that physical activity levels and capacity are not negatively affected by iron status in healthy populations.

Overall, the environmental characteristics and lifestyle behaviours investigated do not seem to be meaningfully associated with donor blood parameters relevant to blood service practice. In addition, we conclude that medium-to-long-term lower iron status (i.e.lower ferritin levels, but still adequate haemoglobin levels) do not translate into differences in habitual physical activity levels and sedentary behaviour.

References

1. Atsma F, Veldhuizen I, Verbeek A, de Kort W, de Vegt F. Healthy donor effect: its magnitude in health research among blood donors. Transfusion 2011; 51(8): 1820-8.

2. Newman BH, Newman DT, Ahmad R, Roth AJ. The effect of whole-blood donor adverse events on blood donor return rates. Transfusion 2006; 46(8): 1374-9.

3. Atsma F, de Vegt F. The healthy donor effect: a matter of selection bias and confounding. Transfusion 2011; 51(9): 1883-5.

4. Beard J, Tobin B. Iron status and exercise. Am J Clin Nutr 2000; 72(2 Suppl): 594S-7S.

5. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. Eur J Epidemiol 2015; 30(7): 543-52.

6. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003; 32(1): 1-22.

7. Moore C, Sambrook J, Walker M, et al. The INTERVAL trial to determine whether intervals between blood donations can be safely and acceptably decreased to optimise blood supply: study protocol for a randomised controlled trial. Trials 2014; 15: 363.

8. Timmer TC, de Groot R, Habets K, et al. Donor InSight: characteristics and representativeness of a Dutch cohort study on blood and plasma donors. Vox Sang 2018.

9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18(6): 499-502.

10. Nigam PK. Serum Lipid Profile: Fasting or Non-fasting? Indian J Clin Biochem 2011; 26(1): 96-7.

11. Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein cholesterol concentrations in the plasma of human subjects as measured in the fed and fasted states. Clin Chem 1988; 34(12): 2456-9.

12. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID, Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992; 38(1): 150-60.

13. Kanias T, Lanteri MC, Page GP, et al. Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-Omics study. Blood advances 2017; 1(15): 1132-41.

14. Chaudhary R, Dubey A, Sonker A. Techniques used for the screening of hemoglobin levels in blood donors: current insights and future directions. [Review]. Journal of Blood Medicine 2017; 8: 75-88.

15. Baart AM, de Kort WL, van den Hurk K, Pasker-de Jong PC. Hemoglobin assessment: precision and practicability evaluated in the Netherlands-the HAPPEN study. Transfusion 2016; 56(8): 1984-93.

16. Chaudhary R, Dubey A, Sonker A. Techniques used for the screening of hemoglobin levels in blood donors: current insights and future directions. J Blood Med 2017; 8: 75-88.

17. van Poppel MN, Chinapaw MJ, Mokkink LB, van Mechelen W, Terwee CB. Physical activity questionnaires for adults: a systematic review of measurement properties. Sports Med 2010; 40(7): 565-600.

18. Dowd KP, Szeklicki R, Minetto MA, et al. A systematic literature review of reviews on techniques for physical activity measurement in adults: a DEDIPAC study. Int J Behav Nutr Phys Act 2018; 15(1): 15.

19. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Act 2008; 5: 56.

20. Hajna S, White T, Panter J, et al. Driving status, travel modes and accelerometer-assessed physical activity in younger, middle-aged and older adults: a prospective study of 90 810 UK Biobank participants. Int J Epidemiol 2019.

21. White T, Westgate K, Wareham NJ, Brage S. Estimation of Physical Activity Energy Expenditure during Free-Living from Wrist Accelerometry in UK Adults. PLoS One 2016; 11(12): e0167472.

22. Barker J, Smith Byrne K, Doherty A, et al. Physical activity of UK adults with chronic disease: cross-sectional analysis of accelerometer-measured physical activity in 96 706 UK Biobank participants. Int J Epidemiol 2019.

23. Cassidy S, Fuller H, Chau J, Catt M, Bauman A, Trenell MI. Accelerometer-derived physical activity in those with cardio-metabolic disease compared to healthy adults: a UK Biobank study of 52,556 participants. Acta Diabetol 2018; 55(9): 975-9.

24. van Nassau F, Mackenbach JD, Compernolle S, de Bourdeaudhuij I, Lakerveld J, van der Ploeg HP. Individual and environmental correlates of objectively measured sedentary time in Dutch and Belgian adults. PLoS One 2017; 12(10): e0186538.

25. Loyen A, Van Hecke L, Verloigne M, et al. Variation in population levels of physical activity in European adults according to cross-European studies: a systematic literature review within DEDIPAC. Int J Behav Nutr Phys Act 2016; 13: 72.

26. Golubic R, May AM, Benjaminsen Borch K, et al. Validity of electronically administered Recent Physical Activity Questionnaire (RPAQ) in ten European countries. PLoS One 2014; 9(3): e92829.

27. Ekelund U, Sepp H, Brage S, et al. Criterion-related validity of the last 7-day, short form of the International Physical Activity Questionnaire in Swedish adults. Public Health Nutr 2006; 9(2): 258-65.

28. Hagstromer M, Bergman P, De Bourdeaudhuij I, et al. Concurrent validity of a modified version of the International Physical Activity Questionnaire (IPAQ-A) in European adolescents: The HELENA Study. Int J Obes (Lond) 2008; 32 Suppl 5: S42-8.

29. World Health Organization. Global recommendations on physical activity for health. Geneva, Switzerland, 2010.

30. Warburton DER, Bredin SSD. Health benefits of physical activity: a systematic review of current systematic reviews. Curr Opin Cardiol 2017; 32(5): 541-56.

31. Sattelmair J, Pertman J, Ding EL, Kohl HW, 3rd, Haskell W, Lee IM. Dose response between physical activity and risk of coronary heart disease: a meta-analysis. Circulation 2011; 124(7): 789-95.

32. Eijsvogels TMH, Thompson PD, Franklin BA. The "Extreme Exercise Hypothesis": Recent Findings and Cardiovascular Health Implications. Curr Treat Options Cardiovasc Med 2018; 20(10): 84.

33. Rigas AS, Sorensen CJ, Pedersen OB, et al. Predictors of iron levels in 14,737 Danish blood donors: results from the Danish Blood Donor Study. Transfusion 2014; 54(3 Pt 2): 789-96.

34. Cable RG, Glynn SA, Kiss JE, et al. Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. Transfusion 2012; 52(4): 702-11.

35. Kotze SR, Pedersen OB, Petersen MS, et al. Predictors of hemoglobin in Danish blood donors: results from the Danish Blood Donor Study. Transfusion 2015; 55(6): 1303-11.

36. Zijp IM, Korver O, Tijburg LB. Effect of tea and other dietary factors on iron absorption. Critical reviews in food science and nutrition 2000; 40(5): 371-98.

37. Cummins S, Curtis S, Diez-Roux AV, Macintyre S. Understanding and representing 'place' in health research: a relational approach. Soc Sci Med 2007; 65(9): 1825-38.

38. Perchoux C, Chaix B, Cummins S, Kestens Y. Conceptualization and measurement of environmental exposure in epidemiology: accounting for activity space related to daily mobility. Health Place 2013; 21: 86-93.

39. Sallis JF, Owen N, Fisher EB. Ecological models of health behavior. 4th ed. San Francisco, U.S.A.: Jossey-Bass; 2008.

40. Booth SL, Sallis JF, Ritenbaugh C, et al. Environmental and societal factors affect food choice and physical activity: rationale, influences, and leverage points. Nutr Rev 2001; 59(3 Pt 2): S21-39; discussion S57-65.

41. Allender S, Foster C, Hutchinson L, Arambepola C. Quantification of urbanization in relation to chronic diseases in developing countries: a systematic review. J Urban Health 2008; 85(6): 938-51.

42. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behav Res Methods Instrum Comput 2004; 36(4): 717-31.

43. Kowalski A, Grant F, Okuku H, et al. Determinants of anemia and iron status among pregnant women participating in the Mama SASHA Cohort Study of Vitamin A in Western Kenya: Preliminary findings. FASEB Journal Conference: Experimental Biology 2014; 28(1 SUPPL. 1).

44. Gupta SK. Intention-to-treat concept: A review. Perspect Clin Res 2011; 2(3): 109-12.

45. Delgado-Rodriguez M, Llorca J. Bias. J Epidemiol Community Health 2004; 58(8): 635-41.

46. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009; 338: b2393.

47. Leipnitz S, de Vries M, Clement M, Mazar N. Providing health checks as incentives to retain blood donors — Evidence from two field experiments. International Journal of Research in Marketing 2018; 35(4): 628-40.