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Restless Legs Syndrome, pica, and iron status in blood donors

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Abstract

BACKGROUND—The association of blood donation related iron deficiency with pica or Restless Leg Syndrome (RLS) remains poorly elucidated. This study evaluated the prevalence of RLS and pica in blood donors completing the REDS-II Iron Status Evaluation (RISE) Study.

STUDY DESIGN AND METHODS—RISE enrolled 2425 blood donors in a prospective cohort study; 1334 donors provided blood samples to characterize iron status and answered a questionnaire inquiring into symptoms of RLS and pica at a final visit after 15–24 months of follow-up. Associations between both conditions and iron status were evaluated.

RESULTS—There were 9% and 20% of donors reporting symptoms of Probable or Probable/Possible RLS, respectively. Iron depletion and donation intensity were not predictive of RLS. Pica was reported by 65 donors (5.5%), half of whom reported daily cravings. Prevalence of pica increased with degree of iron depletion in women (2% in iron replete females, 13% in those with ferritin < 12ng/mL), but not in men. Probable RLS and pica co-expressed in 8 individuals, but no more frequently than expected by chance.

CONCLUSION—RLS and pica have been associated with iron deficiency in non-donor populations. This study indicates a potentially high prevalence of RLS in frequent blood donors but shows no association with iron status or donation intensity. Low iron stores were associated with higher prevalence of pica, but only in females. Furthermore, the results are incompatible with RLS and pica sharing a common pathophysiology.

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Keywords

blood donor; iron depletion; donation interval; pica; Restless Legs Syndrome

INTRODUCTION

Iron depletion as a consequence of repeated blood donation is well-documented.[1] Only recently have comprehensive, longitudinal data been collected that speak to the impact of demographic, behavioral, and genetic factors along with donation intensity upon decrements in serum iron parameters.[2] While iron depletion is thought to contribute to fatigue and cognitive impairment, its clinical correlates are not firmly established. Both acute and chronic iron deficiency interfere with the function of the neurotransmitter dopamine,[3] which is essential to motivation, reward, feeding, sleep, and vigilance.[4] Two common clinical conditions in which iron deficiency has been implicated as an intermediate trait --accounting for the sensitivities of each trait to dopaminergic medications-- are Restless Legs Syndrome (RLS),[5,6] and pica.[7,8]

RLS is a common disorder that manifests as an intense urge to move the legs that is uncomfortable, worsens at rest, is relieved by movement, and that interferes with sleep because of its diurnal preference for the evening and night.[9] Insufficient brain iron has been posited as a universal feature common to both the primary/idiopathic and secondary forms of RLS,[5,6] and genetic variants conferring susceptibility to RLS associate with lower measures of mobilizable iron in a dose-dependent fashion.[10] Nonetheless, iron deficiency is neither necessary nor sufficient to cause RLS.[9] Pica has been described as a “devouring passion” or insatiable urge to consume primarily non-nutritive items, including ice, dirt, clay, raw pasta, and starch.[11,12] An association with iron decrements is extensively documented.[11] Associations of iron deficiency with RLS and pica, two conditions which share in common compulsive urges that are modifiable by dopaminergic drugs, suggests a potential shared etiology. Blood donors represent a population suitable for further delineating the potentially causative associations of iron-deficiency to both RLS and pica, and to examine for the first time the potential inter-relationship between these two clinical conditions. Previous studies, although reporting prevalence of RLS[13,14] and pica[14] that were much higher amongst blood donors, failed to find or examine for a relationship to donation intensity. We therefore examined self-reported RLS-like symptoms and pica behaviors among repeat blood donors at the conclusion of the Retrovirus Epidemiology Donor Study – II (REDS-II) Iron Status Evaluation Study (RISE), and probed for associations between iron status and RLS and pica.

MATERIALS AND METHODS

Donor population

The REDS-II RISE Study has been described elsewhere.[2] Briefly, six blood centers participating in the REDS-II program, sponsored by the National Heart, Lung and Blood Institutes (NHLBI), recruited 2425 donors for multiple follow-up visits over 15–24 months. Donors were recruited into 4 cohorts stratified by gender and recent donation history.

Donors with no prior donation history (FT), or no donations within the last two years (RA) entered the "First-Time/Reactivated" donor cohort (FT/RA), while those who had made 2 (Females) or 3 (Males) whole blood donations in the prior 12 months entered the Frequent Repeat (RPT) cohorts. Information on behavioral, demographic, and other factors was collected at enrollment. When possible, extra plasma samples were collected from all donor visits for hematological and iron assays. Of the 2425 donors enrolled at baseline, 1334 donors (187 FT/RA Females, 149 FT/RA Males, 486 RPT Females, 512 RPT Males) completed a final visit at least 15 months post enrollment. The Final Visit (FV) differed from other follow-up visits by the administration of a questionnaire on use of mineral and vitamin supplements, and symptoms indicative of RLS or pica. This manuscript reports the prevalence of RLS and pica at the time of the FV and characterizes their associations with demographic factors and iron status observed at the same time.

Restless Legs Syndrome in RISE Donors

Under the best of circumstance, the sensitivities and specificities of subjective assessments for RLS do not exceed 0.80 to 0.90[15] due to several clinical conditions that closely mimic RLS.[16] We therefore employed two different screening questions, one with nearly 100% sensitivity and 97% specificity in a sleep disorders population (Q9, Appendix #1),[17] and another with approximately 75% sensitivity and specificity in a general population (Q10, Appendix #2).[10] Donor status was classified as "Probable RLS" if a subject was affected by RLS-like symptoms at least two to four times per month with the additional requirements of worsening while at rest and being relieved by movement (Condition 2; Table in Appendix 1), and that predominated in the evening or at bedtime (Condition 3; Table in Appendix 1). "Possible RLS" applied to individuals who reported symptoms less frequently, or who answered "don't know" as to worsening at rest or relief with movement, but still noted a preponderance of symptoms in the evening or at night (Appendix 1). The classifications of "Probable" or "Possible " RLS were mutually exclusive. Those who answered "No" to both screening questions were classified as not having RLS, as were those who answered affirmatively to one or both screening questions but whose symptoms did not conform to the consensus definition of RLS. Those with information missing for any of the 5 questions or serum iron parameters at FV were excluded from analysis. Of the 1334 donors with a FV, 1166 had complete information on RLS questions and 1145 of these had information on iron status.

Pica in RISE Donors

A capture question asking "*Do you ever crave and regularly eat or chew non-nutritional substances, such as ice, clay, dirt, starch, raw pastas, chalk or coal*?" was used to classify donors as having pica (See Appendix 2). Follow-up questions asked about specific substances and inquired into the frequency and duration of these cravings as well as their responsiveness to blood donation. Due to the challenge of eliciting detail on socially unusual behaviors, donor response to the capture question alone was used to classify subjects as reporting the presence or absence of pica, independent of their follow-up responses. Of the 1334 donors with a FV, 1175 completed the capture question, and 1154 had information on their iron status.

Statistical Analysis

Donors participating in RISE were previously characterized at enrollment (Baseline Visit) and study end (Final Visit) for several variables, including demographic factors, donation intensity, hemoglobin and iron levels, and derived iron outcome measures of *Iron Deficient Erythropoiesis* (IDE) and *Absent Iron Stores* (AIS). IDE was defined as the upper 2.5% of the distribution of log(soluble transferrin receptor/ferritin) in the First Time Male cohort at enrollment and this threshold was applied to both men and women in all four cohorts. AIS was defined as ferritin < 12 ng/mL. Summary measures were presented as means ± SD, median (ferritin), or prevalence (AIS, IDE), stratified by FT-RA/RPT and gender status. To determine whether those who completed RISE were systematically different from those who did not, baseline measures were stratified according to whether donors completed a FV. In logistic regression analysis, unadjusted and adjusted odds ratios for predictors of Probable/Possible RLS, Probable RLS only, and pica were developed using PROC LOGISTIC in SAS, v. 9.2. For adjusted odds ratios, independent variables were included if they had previously been shown to relate to the outcomes of interest (such as higher reported prevalence of RLS in females) or if they were predictive of an individual's iron status (age, body mass, use of supplemental iron). Models were also evaluated that estimated the explanatory value of donation intensity and changes in iron status (across replete/IDE/AIS) from enrollment to FV. Missing values for independent variables were included (using missing as an additional category), but were not reported in summary tables.

RESULTS

Donors completing RISE

As discussed in detail elsewhere,[2] 1334 of 2425 donors (55%) who enrolled in RISE returned for a FV (Table 1). While a large proportion of donors in both the FT/RA (70 to 79%) and Frequent (>95%) cohorts returned for at least one visit following enrollment, the percentages who made a FV were considerably smaller, from 37–39% for the FT/RA male and female cohorts, to 63–67% for the RPT male and female cohorts. Table 1, which characterizes the 4 RISE cohorts at baseline –stratified on whether they completed the study with a FV – shows that on many measures, including hemoglobin and indicators of iron status, those who completed RISE were very similar at enrollment to those who did not make a FV. On average, within each of the 4 cohorts, those completing the FV were older by 6–7 years, more likely to be taking supplemental iron at enrollment, and had higher donation intensity in the prior 12- and 24-month period prior to enrollment (for the RPT cohorts). In addition, the FT donor cohorts showed a significant increase in the prevalence of IDE and AIS between enrollment and their FV, while this did not occur to a substantial degree in the RPT cohorts.[2]

Restless Legs Syndrome in RISE Donors

Across 1166 donors completing the RLS questions at FV, 9% were classified as reporting Probable RLS and an additional 11% were classified as Possible RLS; hence, a total of 20% had Probable/Possible RLS. Both Probable and Possible RLS were nearly twice as prevalent in females as in males, and no association with iron status was evident (Table 2). Table 3 reports Odds Ratios for RLS for several demographic factors and for iron status, with

unadjusted ORs presented from univariate analysis and adjusted ORs from multivariable logistic regression models. Across all models, gender is the only predictor with a consistent and statistically significant association with RLS, with risk in females roughly twice that of males for Probable/Possible RLS (OR = 2.19, 95% CI 1.52, 3.14) and for Probable RLS (OR = 2.08, 95% CI 1.26, 3.46) in multivariable analysis. Younger age (< 40) is associated with a risk that is at least 50% lower compared to 40 to 49 year olds, who are statistically indistinguishable from those 50 and older. The apparent lower risk of younger donors is consistent for both Probable/Possible and Probable RLS, though the association does not reach statistical significance in the latter case. Neither weight, use of supplemental iron, nor iron status is associated with either Probable or Possible RLS categories in unadjusted or adjusted analyses. Alternate models that added measures for recent donation intensity, that substituted donation intensity for iron status, or that evaluated *changes* in iron status across a spectrum of replete/IDE/AIS did not improve model fit or predictive power. Because RLS prevalence is highest in those with Northern European origins,[9] genetic variants conferring susceptibility for RLS are under-represented in sub-Saharan Africans.[18] In addition, there are distinct racial differences in hematological measures and iron parameters;[19] therefore, we repeated these analyses on the 86% of subjects comprising the total RISE population who self-reported as Caucasian. Results were not affected by restricting analyses to Caucasians. Associations of iron status with greater frequency of RLS symptoms (5 or more times per month) were also absent in both unadjusted and adjusted analyses.

Pica in RISE Donors

Pica was reported by 5.5% (65 of 1175) of RISE donors who answered the capture question (Table 2). A clear trend was seen between iron status and pica in females, with reported prevalence increasing from 2% in iron replete females to 6% in those with IDE, to 13% of those with AIS. No such trend was seen in males, with higher prevalence of pica (6%) reported from iron replete males and those with AIS, and the lowest (1%) from those with IDE. In multivariable models (Table 3), those age 50 or older were associated with 65–70% lower risk, with those less than 40 years old statistically equivalent to those who were 40–49 years old. Despite losing a proportionally larger share of blood with each donation, those whose weight was < 150 pounds had a statistically significant lower risk for pica compared to those 150–174 pounds (OR = 0.33, 95% CI 0.14, 0.79). A gender by iron status interaction term was statistically significant, with no clear trend by iron status in males but with the estimated risk for pica being 3 times greater for females with IDE and 8 times greater for those with AIS compared to females who were iron replete (p=.0002). As with the RLS models, donation intensity did not improve the model's discriminative power. Of those reporting pica, half claimed that their cravings occurred daily. Eleven donors reported the actual item(s) they consumed, with raw pasta (N=5) and starch (5) being most frequently reported, followed by clay, ice, and raw oats (1 each; 2 donors reported consumption of 2 items).

Coincidence of RLS and pica

RLS and pica were both reported at relatively high levels compared to population norms, but did not co-occur in RISE donors in a statistically significant manner. This was equally true

for Probable/Possible RLS (17 donors; p=0.17), Probable RLS (8 donors; p= 0.31), and RLS with high frequency of symptoms (5 donors, p=0.25).

DISCUSSION

Despite the causal association between repeated blood donation and low iron stores, little documentation exists of the attendant clinical consequences. It might be the case that iron depletion in blood donors is of limited clinical significance, if those whose iron stores become depleted through repeated phlebotomy do not perceive any adverse outcomes. Alternately, the impact might be self-limiting since donors who do experience symptoms might take action to avoid adverse consequences. Symptom abrogation could occur through a variety of means including supplemental iron ingestion, lengthening intervals between donations, or discontinuing blood donation altogether. What cannot be disputed is that intermediate (IDE) and advanced (AIS) iron depletion are common outcomes in repeat blood donors in the REDS-II RISE study, and that the strongest predictor of IDE and AIS is frequency of donation.

Though the primary intent of RISE was to characterize the prevalence, incidence, and predictors of iron deficiency in blood donors, the study also collected clinical information in order to assess for the potential consequences of the resultant iron deficiency. Assessed only at the FV, symptoms compatible with Probable RLS were reported by 9% and with Probable/Possible RLS by 20% of RISE donors, respectively. As detailed elsewhere[2] and shown in Table 1, these results occurred in donor cohorts with a high prevalence of iron depletion (near or surpassing 50% prevalence of IDE in 3 of the 4 cohorts) and recent donation intensity that is considerably higher than the “average” donor. Though several-fold higher than reported figures for weekly (5%) or “medically significant” (2.7%) RLS symptoms in a recent report drawn from the general population,[20] RLS prevalence between 9 and 20% is consistent with other studies in blood donor populations.[13,14] In RISE, as in these earlier studies, RLS prevalence nearly twice that of the general population in a cohort enriched for iron deficiency (e.g., blood donors), does not require that such an association holds at the individual level, or that it is necessarily causative. The most parsimonious explanation for our observing that iron deficiency is insufficient by itself to associate with RLS, is that RLS is a complex trait influenced by a number of factors including gender and age, as demonstrated here and elsewhere, as well as many environmental and genetic factors.[9] Iron status appears to be only one element of one or more causal pathways that can lead to a RLS phenotype, since iron deficiency as manifest in serum parameters is also not necessary for RLS symptom expression (*e.g.*, we observed Probable RLS in 49 subjects classified as iron replete).[6] Ongoing and future research should help elucidate the nature and magnitude of the association between iron and RLS, in blood donor populations and more generally.

The results for pica in RISE donors are more suggestive, but nonetheless, inconclusive. A dose-response relationship between iron status and prevalence of pica was observed in female RISE donors, with those who are iron replete being 8 times less likely to report pica than those who have AIS at FV. In male donors, no such association was found. This apparent gender difference may be real (albeit unexplained) or may derive from the presence

of relatively sparse outcomes in a multivariable logistic model, or potentially represent a differential in accuracy of information elicited from male and female RISE donors. In any case, this mixed result, while unexplained, is consistent with the only other report of which we are aware that has measured iron status and assessed pica in blood donors, which also found an association between pica and iron status in females but not in male donors.[14] Neither our study nor the earlier one on pica in blood donors reports a higher co-occurrence of pica and RLS than would be expected by chance. While intriguing given the paucity of clinical data associated with iron stores in blood donors, these results need to be interpreted cautiously. There are several limitations to our study. The RISE study was not designed, or powered, to test hypotheses relating to clinical outcomes. With both RLS and pica having been assessed only at the FV, prevalence is the only measure of association available. Estimates of incidence, if available, would add valuable detail to these findings, as would finer temporal resolution regarding putative associations, including age of onset, whether initial onset of symptoms preceded blood donation, symptom duration, and whether symptoms fluctuate as a function of donation activity and serum iron parameters. Furthermore, accuracy of RLS and pica diagnoses were not validated in RISE, which can be problematic due to several mimics of RLS not easily discernable by self report [15,16] That being said, the tools used to assign RLS affliction status here demonstrate discriminative power sufficient to discern genetic susceptibility factors of modest effect sizes (i.e., ORs of > 1.5 per risk allele).[10] Diagnosis of pica is also challenging given the stigma attached to socially unusual behaviors,[11] albeit perhaps less so if ice is the primary non-nutritive substance ingested. Only ~20% of RISE donors who reported pica specified what item(s) they craved and consumed, despite the questionnaires being self-administered rather than performed by oral interview. Almost half of those admitting to this behavior acknowledged that the cravings were intense as reflected in their daily occurrence. This suggests that the phenomenon is genuine, even if more detailed disclosures might require additional investigator follow-up not available for RISE. The findings reported here increase our appreciation for the fact that RLS and pica are experienced by a significant number of frequent blood donors. This is particularly true of older women and RLS, and younger females in the case of pica. Further research on these and other outcomes associated with low iron stores could provide significant value in terms of characterizing the problem of iron depletion of blood donors and developing appropriate responses to protect donor health and quality of life. Moreover, such studies may yield important insights into the interactions between measures of peripheral iron status, brain dopamine network “tone,” and the pathophysiologies underlying behaviors which share potent “urge” components.

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Appendix 1: Classification of Possible and Probable RLS

Q9: When you try to relax in the evening or sleep at night, how often do you have unpleasant, restless feelings in your legs that can be relieved by walking or movement?

- **a.** Never
- **b.** Rarely (once a month or less)
- **c.** Sometimes (2 to 4 times a month)
- **d.** Often (5 to 15 times a month)
- **e.** Very often (16 or more times a month)

Q10: How often do you experience a strong urge to move your legs usually accompanied or caused by unpleasant sensations in your legs – for example, restlessness, creepy-crawly, or tingly feelings?

- **a.** Never
- **b.** Rarely (once a month or less)
- **c.** Sometimes (2 to 4 times a month)
- **d.** Often (5 to 15 times a month)
- **e.** Very often (16 or more times a month)

NB: If answers to both Q9 and Q10 were “never” donors were instructed to skip questions 11–13:

Q11: Is the urge to move your legs or are the unpleasant sensations partially or totally relieved by movement, such as walking or stretching?

- **a.** Yes
- **b.** No
- **c.** Don’t know

Q12: Does the urge to move your legs begin, or do the unpleasant sensations begin or worsen, during periods of rest or inactivity such as when sitting or lying down?

- **a.** Yes
- **b.** No
- **c.** Don’t know

Q13: At what times is the urge to move your legs or the unpleasant sensations most bothersome?

- **a.** in the morning (before noon)
- **b.** in the afternoon (before supper)
- **c.** in the evening (after supper)

d. at night while sleeping

e. no difference by time of day

Conditions required to be classified as probable or possible RLS

	Condition 1	Condition 2	Condition 3
Probable RLS	Q9=c, d, or e OR Q10=c, d, or e	Q11 = Yes and Q12=Yes	Q13 = c or d
Possible RLS	Q9=b, c, d, or e OR Q10=b, c, d, or e	Q11 = Yes and Q12=Yes, OR Q11 = Don't Know and Q12 = Yes OR Q11 = Yes and Q12 = Don't Know	Q13 = c or d

Note: Probable RLS was evaluated first; if those criteria were not met the donor was evaluated for Possible RLS. Probable/Possible RLS includes persons with either Probable or Possible RLS

Appendix 2: Classification of Pica

Q14: Do you ever crave and regularly eat or chew non-nutritional substances, such as ice, clay, dirt, starch, raw pastas, chalk or coal?

☐Yes

☐No (**if No, Stop and Skip to END STATEMENT**)

Q15: Which non-nutritional substances do you consume (check all applicable)?

☐Ice

☐Clay/Dirt

☐Starch

☐Raw Pasta

☐Chalk

☐Coal (charcoal)

☐Other _____

Q16: How often do you consume one or more of the above substance(s)?

☐Daily

☐Weekly

☐Monthly

Q17: How does blood donation impact these cravings?

☐Has no impact

☐Cravings increase after each blood donation

☐Cravings decrease after each blood donation

Q18: How long do your cravings last?

☐Less than 1 week

☐1 to 3 weeks

☐1 month or more

☐Continual Cravings

Q19: Are you currently experiencing this type of craving?

☐Yes

☐No

Table 1

Characterization of RISE donors at baseline (N=2425) and final visits (N=1334)

	FT/RA Female			FT/RA Male			Frequent Female			Frequent Male		
	Baseline visit for those with No FV (N=294)	Baseline visit for those with FV (N=187)	Final Visit (N=187)	Baseline visit for those with No FV (N=258)	Baseline visit for those with FV (N=149)	Final Visit (N=149)	Baseline visit for those with No FV (N=283)	Baseline visit for those with FV (N=486)	Final Visit (N=486)	Baseline visit for those with No FV (N=256)	Baseline visit for those with FV (N=512)	Final Visit (N=512)
Age	36.8 ± 14.8	44.0 ± 15.2	45.5 ± 15.3	35.0 ± 14.5	41.6 ± 16.2	43.2 ± 16.3	45.2 ± 14.6	51.2 ± 12.3	52.8 ± 12.2	47.5 ± 14.8	53.2 ± 13.3	54.8 ± 13.2
Weight	159 ± 36	162 ± 39	NA	193 ± 38	198 ± 39	NA	165 ± 37	159 ± 33	NA	199 ± 37	198 ± 35	NA
Fe supplementation	42%	44%	34%	22%	30%	21%	47%	55%	46%	29%	34%	25%
RBC donations last 24m	0 ± 0	0 ± 0	3.7 ± 1.9	0 ± 0	0 ± 0	4.6 ± 2.2	4.2 ± 2.3	5.7 ± 2.5	6.5 ± 2.4	6.1 ± 2.8	7.5 ± 2.5	8.4 ± 2.3
RBC donations last 12m	0 ± 0	0 ± 0	1.6 ± 1.4	0 ± 0	0 ± 0	2.1 ± 1.6	2.4 ± 1.2	3.2 ± 1.2	2.7 ± 1.5	3.4 ± 1.4	3.9 ± 1.1	3.7 ± 1.5
Hb (g/dL)	13.3 ± 0.9	13.4 ± 1.0	13.2 ± 1.2	15.2 ± 1.2	14.9 ± 1.0	14.6 ± 1.3	13.1 ± 0.9	13.2 ± 1.0	13.1 ± 1.2	14.5 ± 1.2	14.5 ± 1.1	14.4 ± 1.5
Ferritin (ng/mL)	36	41	22	109	105	45	20	18	18	26	25	25
AIS (%)	7.5%	4.8%	20.4%	0%	0%	7.7%	27.2%	27.0%	27.3%	17.6%	15.8%	18.1%
IDE (%)	27%	22%	51%	2.3%	2.7%	20%	64%	67%	62%	49%	48%	47%

* Mean ± SD

** Median

Table 2

Prevalence of RLS and pica by iron status at RISE Final Visit (%)

Outcome	All donors (N=1145 of 1334 with FV) *	Females			Males		
		Replete (N=240 of 271 with FV) *	IDE without AIS (N=198 of 221 with FV) *	AIS (N=151 of 168 with FV) *	Replete (N=332 of 384 with FV) *	IDE without AIS (N=131 of 163 with FV) *	AIS (N=93 of 103 with FV) *
Probable/Possible RLS	20.0%	24.2%	27.8%	25.2%	14.2%	12.2%	15.1%
Probable RLS	9.0%	12.1%	10.6%	12.6%	6.0%	6.1%	7.5%
Pica	5.5%	2.0%	6.0%	13.3%	5.8%	0.8%	6.4%

* 1334 donors completed a FV; data shown are for 1145 who completed questions on both RLS and pica and had iron values. The 189 donors whose data are not shown here had missing data for one or more of RLS (N=168), pica (N=159), or iron assays (N=24).

Table 3

Unadjusted and Adjusted Odds Ratios for Possible Predictors of RLS and Pica, Odds Ratio and 95%CI

	Probable/Possible RLS+		Probable RLS+		Pica+	
	Unadjusted OR	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**
Age	**p=.001**	**p=.007**	**p=.09**	**p=.21**	**p=.0003**	**p<.0001**
< 30	0.36 (0.19, 0.70)	0.40 (0.20, 0.78)	0.44 (0.18, 1.10)	0.51 (0.20, 1.28)	1.36 (0.66, 2.83)	1.74 (0.80, 3.78)
30–39	0.42 (0.21, 0.82)	0.42 (0.22, 0.83)	0.33 (0.11, 0.98)	0.34 (0.11, 1.00)	1.17 (0.53, 2.59)	1.23 (0.54, 2.80)
40–49	Ref	Ref	Ref	Ref	Ref	Ref
50–59	0.97 (0.66, 1.42)	1.00 (0.68, 1.48)	0.88 (0.52, 1.50)	0.88 (0.51, 1.51)	0.38 (0.18, 0.79)	0.36 (0.17, 0.76)
60+	0.76 (0.51, 1.13)	0.80 (0.53, 1.20)	0.92 (0.54, 1.56)	0.97 (0.56, 1.67)	0.29 (0.13, 0.64)	0.30 (0.13, 0.69)
Gender	**p<.0001**	**p<.0001**	**p=.0009**	**p=.005**	**p=.17**	
Female	2.09 (1.55, 2.82)	2.19 (1.52, 3.14)	2.01 (1.32, 3.07)	2.08 (1.26, 3.46)	1.42 (0.85, 2.37)	Interaction with iron shown below
Male	Ref	Ref	Ref	Ref	Ref	
Weight	**p=.09**	**p=.70**	**p=.22**	**p=.54**	**p=.29**	**p=.006**
< 150	1.54 (1.03, 2.30)	1.20 (0.79, 1.83)	1.80 (1.01, 3.19)	1.39 (0.77, 2.52)	0.52 (0.23, 1.19)	0.33 (0.14, 0.79)
150–174	Ref	Ref	Ref	Ref	Ref	Ref
175–199	0.96 (0.63, 1.48)	1.11 (0.71, 1.74)	1.18 (0.64, 2.20)	1.29 (0.68, 2.44)	1.06 (0.53, 2.09)	1.34 (0.63, 2.79)
200+	1.11 (0.74, 1.67)	1.37 (0.88, 2.14)	1.34 (0.75, 2.41)	1.73 (0.92, 3.25)	1.11 (0.58, 2.13)	2.00 (0.95, 4.19)
Supplemental iron	**p =.30**	**p=.16**	**p=.09**	**p=.49**	**p=.75**	**p=.21**
Yes	1.19 (0.86, 1.63)	1.04 (0.74, 1.45)	1.46 (0.95, 2.27)	1.32 (0.84, 2.07)	1.09 (0.64, 1.86)	1.49 (0.84, 2.64)
No	Ref	Ref	Ref	Ref	Ref	Ref
Iron status	**p =.42**	**p=.81**	**p=.63**	**p=.78**	**p=.001**	
Replete	Ref	Ref	Ref	Ref	Ref	Interaction with gender shown below
IDE without AIS	1.22 (0.87, 1.72)	1.14 (0.80, 1.62)	1.03 (0.64, 1.67)	0.99 (0.61, 1.63)	0.94 (0.47, 1.87)	
AIS	1.21 (0.83, 1.75)	1.11 (0.76, 1.64)	1.27 (0.77, 2.10)	1.23 (0.73, 2.07)	2.74 (1.54, 4.87)	
			Gender by Iron status interaction		**P<.0001**	**p=.0002**
			Male Replete vs. Female Replete		2.98 (1.10, 8.09)*	1.92 (0.68, 5.4)
			Male IDE vs Male Replete		0.12 (0.02, 0.94)*	0.12 (0.02, 0.95)*
			Male AIS vs Male Replete		1.11 (0.43, 2.87)	1.28 (0.48, 3.38)

	Probable/Possible RLS[+]		Probable RLS[+]		Pica[+]	
	Unadjusted OR	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**
			Female IDE vs. Female Replete		3.10 (1.07, 8.96)*	3.18 (1. 08, 9.39)*
			Female AIS vs. Female Replete		7.42 (2.72, 20.22)**	8.16 (2.90, 22.96)**

* $p < .05$

** $p < .0001$

+ Adjusted models for RLS include 1166 donors and that for pica includes 1175 donors.