

THE EFFECTS OF IRON DEPLETION WITHOUT ANEMIA ON TRAINING AND
PERFORMANCE IN FEMALE COLLEGIATE ROWERS

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THE EFFECTS OF IRON DEPLETION WITHOUT ANEMIA ON TRAINING AND PERFORMANCE IN FEMALE COLLEGIATE ROWERS

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This dissertation investigated the effects of iron depletion without anemia (IDNA) on physical performance and training in female collegiate rowers. In a cross-sectional study, 165 rowers were screened for iron status at the beginning of a competitive season (10% anemic, 30% IDNA, Hgb>12.0, Hgb>12.0 g/dL and ferritin <20 µg/L). IDNA rowers reported 2K times that were 21 seconds slower compared to rowers with normal iron status (p=0.004). During the first week of training, 48 rowers (n=24 IDNA) had their physical performance assessed (VO₂peak, 4K time, gross energetic efficiency) and recorded their training regimen. Compared to rowers with normal iron status, IDNA rowers trained ~10 minutes/d less (p=0.02), and had a 0.3 L/min lower VO₂peak (p=0.03). Less highly-trained rowers with poor iron status had a lower VO₂peak (-0.32 L/min, p=0.02), and were less energetically-efficient (-1.7%, p=0.09) compared to more highly-trained rowers with poor iron status.

In a randomized controlled trial, 43 rowers received 100 mg/d FeSO₄ (n=22) or placebo (n=21) for 6 weeks, and completed daily training logs. Iron status, performance, and training quality were assessed at baseline and 6 weeks. Thirty-one rowers (n=15 iron, 16 placebo) completed the trial. Rowers supplemented with iron improved their body iron stores (log ferritin, total body iron, p=0.07), and those with most depleted stores at baseline improved the most. Blood lactate concentration during the first 2000m of a 4K TT and 5 min post-recovery was significantly lower in

the iron group ($p < 0.01$), and rowers in the iron group had a greater improvement in work efficiency ($p = 0.15$) compared to placebo. Additionally, the energetic efficiency of those rowers with poorer baseline stores (ferritin $< 20 \mu\text{g/L}$) benefitted more from supplementation. Finally, rowers in the iron group had an improved training quality score ($p = 0.03$) compared to those in the placebo group.

We conclude that iron status should be screened at the beginning of a training season, and that iron supplementation ($\sim 15 \text{ mg iron/d}$) improves iron stores in female rowers during training, especially in the most depleted. The iron status of those with marginal/low stores should be monitored to prevent detrimental effects on training and performance.

BIOGRAPHICAL SKETCH

Diane M. DellaValle was born and raised in Pennsylvania. As early as elementary school, Diane became interested in using food and nutrition to communicate scientific principles. Additionally, as a high school athlete, she was interested in sports nutrition and learning about the effects of food and nutrients on athletic performance. Diane went on to major in Nutrition and Dietetics at Marywood University in Scranton, PA. She completed the coordinated didactic and internship program mentored by Dr. Marianne Borja, who fostered Diane's interest in research. After graduating from Marywood and earning her certification of Registered Dietitian, Diane went on to pursue her MS in Nutritional Sciences from the Pennsylvania State University in University Park, PA where she worked under the guidance of Dr. Barbara Rolls studying obesity, weight management, and food intake behaviors in humans. After Penn State, Diane came to Cornell University to work as a Research Nutritionist in the Division of Nutritional Science's Human Metabolic Research Unit, and shortly thereafter, enrolled in DNS's PhD program under the mentorship of Dr. Jere Haas.

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TABLE OF CONTENTS

Biographical sketch	iii
Acknowledgements	iv
Table of contents	vi
List of tables	x
List of figures	xiii
List of formulas	xv
List of abbreviations	xvi
Chapter 1. Introduction.....	1
Specific aims and hypotheses	2
References	6
Chapter 2. Literature review	7
Iron absorption and balance	7
Iron requirements	11
Iron supplementation	14
Prevalence of iron deficiency.....	16
Assessment of iron status.....	16
Relationship between iron status, physical performance and physical activity.....	23
Training.....	30
Rowing.....	34
Conceptual framework of current study	38
References.....	41
Chapter 3. General methods	52
IRB approval and subject recruitment	53
Data collection	55
Analytical approach	61
References.....	66

Chapter 4. Impact of iron depletion without anemia on trained endurance athletes at the beginning of a competitive season: a study of female collegiate rowers in central NY state	67
Abstract	67
Introduction.....	68
Methods.....	69
Results.....	72
Discussion.....	76
Conclusion	77
References.....	79
 Chapter 5. How does iron status affect endurance performance and training in female collegiate rowers?.....	82
Abstract	82
Introduction.....	84
Methods.....	85
Results.....	93
Discussion.....	106
Conclusion	111
References.....	112
 Chapter 6. Iron supplementation improves iron status during training in non-anemic female rowers	118
Abstract	118
Introduction.....	119
Methods.....	121
Results.....	127
Discussion.....	138
Conclusion	141
References.....	143

Chapter 7. Iron supplementation improves lactate response and energetic efficiency after training in non-anemic female rowers.....	148
Abstract	148
Introduction.....	150
Methods.....	152
Results.....	162
Discussion.....	177
Conclusion	180
References.....	181
Chapter 8. Iron supplementation improves training quality in non-anemic female rowers	186
Abstract.....	186
Introduction.....	188
Methods.....	189
Results.....	198
Discussion.....	206
Conclusion	210
References.....	211
Chapter 9. General discussion	215
Summary of results	215
Critique of validity	217
Plausability.....	232
Discussion of major findings	237
Iron status	237
Physical performance	241
Training quality.....	248
Recommendations.....	250
Future research.....	252
Conclusions.....	254

References.....	257
Appendices	262
Appendix 1. Validation of training measure: quantification of rowers’ training load using the session RPE method	263
Introduction.....	263
Methods.....	263
Results.....	270
Discussion	276
References.....	279
Appendix 2. Consent Forms	282
Appendix 3. Health and demographic questionnaire.....	288
Appendix 4. Female athlete screening tool (FAST)	294
Appendix 5. Eating Inventory (EI)	296
Appendix 6. Iron-specific food frequency questionnaire	298
Appendix 7. Food record	300
Appendix8 . LTPA and 2K personal record (PR).....	302
Appendix 9. Iron status report	304
Appendix10 .Body composition and lab testing report	306
Appendix11. Daily training log	309
Appendix 12. Exercise test data collection forms.....	312

LIST OF TABLES

Table 2.1. Iron content of selected iron-rich foods	14
Table 2.2. Iron-dependent enzymes.....	25
Table 3.1. Effect sizes of outcome variables used to calculate sample size.....	66
Table 4.1 Iron status of non-anemic female rowers between schools at the beginning of a training season	73
Table 5.1 Anthropometry and body composition of non-anemic rowers at the beginning of a training season	94
Table 5.2 Rowing experience and dietary characteristics of subjects at the beginning of a training season	95
Table 5.3 Iron status of non-anemic rowers at the beginning of a training season	96
Table 5.4 Physical performance of non-anemic rowers during the 4K TT the beginning of a training season	97
Table 5.5 Training of non-anemic rowers at the beginning of a training season	99
Table 5.6 Simple correlations between physical performance and iron status.....	100
Table 5.7 Simple correlations between physical performance and training.....	100
Table 5.8 Physical performance outcomes during 4K TT, adjusted for covariates ...	101
Table 5.9 Regression models to test the effects of iron status on VO ₂ peak.....	102
Table 5.10 Regression models to test the effects of iron status on 4K TT time.....	102
Table 5.11 Regression models to test the effects of iron status on gross EF	103
Table 6.1 Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (entire sample).....	128
Table 6.2 Compliance as measured by training log days recorded (entire sample) ...	128
Table 6.3 Iron status before and after treatment (entire sample).....	129
Table 6.4 Regression models to test effects of iron supplementation on change in iron stores (entire sample).....	130
Table 6.5 Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (IDNA sample).....	132
Table 6.6 Compliance as measured by training log days recorded (IDNA sample) ..	132

Table 6.7 Iron status before and after treatment (IDNA sample).....	133
Table 6.8 Regression models to test effects of iron supplementation on change in log sFer (IDNA sample)	134
Table 6.9 Regression models to test effects of iron supplementation on change in sTfR (IDNA sample)	134
Table 6.10 Regression models to test effects of iron supplementation on change in TBI (IDNA sample)	135
Table 7.1 Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (entire sample).....	163
Table 7.2 Compliance as measured by training log days recorded (entire sample) ...	163
Table 7.3 Physical performance measures before and after 6 weeks of training and treatment (entire sample).....	165
Table 7.4 Regression models to test effects of iron supplementation on change in gross EF after 6 weeks of training and treatment (entire sample)	166
Table 7.5 Regression models to test effects of change in log sFer on change in gross EF after 6 weeks of training and treatment (entire sample)	168
Table 7.6 Physical performance measures before and after 6 weeks of training and treatment (IDNA subgroup)	170
Table 7.7 Regression models to test the effects of iron treatment on change in gross EF after 6 weeks of training and treatment (IDNA subgroup).....	171
Table 7.8. Regression models to test the effects of change in log sFer on change in gross EF after 6 weeks of training and treatment (IDNA subgroup)	172
Table 7.9 Blood lactate concentration (mmol/dL) during 1K segments of 4K TT before and after 6 weeks of training and treatment (entire sample)	175
Table 8.1 Rotated factor loadings of training variables used to compute training quality score.....	197
Table 8.2 Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (entire sample).....	198
Table 8.3 Compliance as measured by training log days recorded (entire sample) ...	199
Table 8.4 Training quality characteristics before and after 6 weeks of training and treatment	200
Table 8.5 Regression models to test the effects of iron supplementation on change in training quality after 6 weeks of training and treatment.....	204
Table A.1 Physical performance during VO ₂ peak test (validation study)	270

Table A.2 Laboratory workout simulations (validation study)	271
Table A.3 Training sessions outside of lab (validation study)	273
Table A.4 Training loads according to session type (validation study)	274

LIST OF FIGURES

Figure 2.1: Effect of iron deficiency on physical activity	24
Figure 2.2: Improvements in time for moderately-trained women to complete a 15 K TT	28
Figure 2.3: Concept 2 rowing ergometer and output monitor	36
Figure 2.4: Conceptual framework for current study	40
Figure 3.1: Map of recruitment area.....	54
Figure 3.2: Timeline and flow of subjects through supplementation trial	58
Figure 4.1 The impact of IDNA on rowers' reported 2K performance.....	75
Figure 5.1 Visual analog scale.....	88
Figure 5.2 Lactate concentration between normal and IDNA rowers during the 4K TT at the beginning of a training season	98
Figure 5.3 Time to complete a 4K TT for low and high trainers according to iron status group (normal vs depleted).....	104
Figure 5.4 VO ₂ peak for low and high trainers according to iron status group (normal vs depleted).....	105
Figure 5.5 Gross EF for low and high trainers according to iron status group (normal vs depleted).....	105
Figure 6.1 Relationship between log sFer at baseline and change in log sFer (entire sample)	131
Figure 6.2 A and B. Relationship between supplement consumed and change in log sFer (A) entire IDNA sample and B) IDNA with >50% compliance)	136
Figure 7.1 A and B. Relationship between dose of supplement consumed and change in gross EF (A): complete sample; B: subgroup consuming >50% of dose	167
Figure 7.2. Relationship between change in log sFer and change in gross EF by iron consumed group in IDNA subgroup.....	173
Figure 7.3 Relationship between change in log sFer and change in gross EF by training group.....	174
Figure 7.4 Lactate concentration as a percent of maximal lactate at the end of the 6-weeks trial between treatment groups (entire sample)	176

Figure 8.1 Timeline and flow of subjects through iron supplementation trial	192
Figure 8.2 Visual analog scale used for daily training questions	196
Figure 8.3 Estimated means of training quality score during the 6-week trial.....	201
Figure 8.4 Relationship between change in training quality score and change in gross energetic efficiency in IDNA rowers according to treatment group	203
Figure 8.5 Relationship between baseline training quality score and change in training quality score according to treatment group	205
Figure 9.1 Relationship between change in training quality score and change in gross energetic efficiency between treatment groups. A: complete sample; B: subgroup of rowers with IDNA at baseline	225
Figure 9.2 Regression analysis to test for mediation.....	226
Figure 9.3 Relationship between supplemental iron intake (mg/kg) to change in total body iron (mg/kg) in A) all iron-supplemented rowers and B) iron-supplemented rowers with IDNA at baseline	234
Figure 9.4 Relationship between change in fat-free mass (kg) and change in soluble transferrin receptor (mg/L) after 6 weeks of treatment	240
Figure A.1 Visual analog scale used for daily training questions (validation study).	269
Figure A.2. Graphic example of typical HR-VO ₂ curve (validation study).....	270
Figure A.3. A and B, Graphic examples of Easy and Hard workout simulations used in validation study (validation study)	272
Figure A.4 A and B. Training load distribution quantified by VAS intensity rating and TRIMP (validation study).....	274
Figure A. 5 A and B. Relationship between training load calculated using absolute and relative methods (validation study)	275
Figure A.6. Relationship between training session duration and VAS intensity rating according to training session type (validation study)	276

LIST OF FORMULAS

Formula 2.1 Calculation of total body iron stores	21
Formula 5.1 Work output	92
Formula 5.2 Energy expenditure	92
Formula 5.3 Gross energetic efficiency.....	92
Formula 5.4 Deviation from work rate prescription.....	92
Formula 7.1 Work output	161
Formula 7.2 Energy expenditure	161
Formula 7.3 Gross energetic efficiency.....	161
Formula 7.4 Deviation from work rate prescription.....	161

LIST OF ABBREVIATIONS

AGP – alpha-1-acid glycoprotein	RCT – randomized, double-blind, placebo-controlled trial
ANOVA – analysis of variance	RDA – recommended daily allowance
APP – acute phase protein	RPE – rating of perceived exertion
APR – acute phase response	sessionRPE – training load (time x intensity)
ATP – adenosine triphosphate	sFer – serum ferritin
BIA – bioelectrical impedance analysis	spm – strokes per minute
Bpm – beats per minute	ST, FT – slow-twitch, fast-twitch
CRP – C-reactive protein	sTfR – soluble transferrin receptor
EAR – estimated average requirement	TBI – total body iron
EF - efficiency	TCA – tri-carboxylic acid cycle
ETC – electron transport chain	TF - transferrin
Fe - iron	TS – transferrin saturation
FEP – free erythrocyte protoporphorin	TT – time trial
FeSO ₄ – ferrous sulfate	TUL – tolerable upper limit
Hgb - hemoglobin	Tx - treatment
ID – iron deficiency/deficient	VAS – visual analog scale
IDA – iron deficiency anemia	VO ₂ max, VO ₂ peak – maximal oxygen consumption
IDNA – iron depletion without anemia	W - watts
K - kilometers	WR – work rate
LTPA – leisure-time physical activity	WR Rx – work rate prescription
NCAA – National Collegiate Athletic Association	
O ₂ - oxygen	
PR – personal record/best	

CHAPTER 1

INTRODUCTION

Iron deficiency (ID) is the most common nutrient deficiency in the United States, affecting 13% of pre-menopausal women, and approximately 30% of physically-active women (1, 2). Iron deficiency anemia (IDA) is clinically defined as hemoglobin (Hgb) less than 12.0 g/dl. Iron depletion without anemia (IDNA), or low iron stores, is defined as Hgb greater than 12.0 g/dl and serum ferritin (sFer) less than 20.0 µg/L. Female athletes are at higher risk of IDNA due to their menstrual status, poor dietary intake, and high training volume and intensity (3). Consequences of IDNA that may be relevant to athletes include reduced work capacity, endurance, and energetic efficiency (4-6); and increased local muscle fatigue (7). The mechanism by which IDNA affects endurance and physical performance remains unclear, and the functional consequences of IDNA are not fully understood in trained individuals, as studies to examine these relationships have been underpowered (8, 9).

Our lab has previously reported the effects of iron deficiency on physical performance in untrained, IDNA women adapting to an aerobic training program. Hinton et al (5) showed that the effect of iron supplementation on physical performance was mediated by changes in iron status (sFer), and concluded that IDNA reduces the potential benefits of aerobic training on endurance. In that study, subjects who were supplemented with iron for 6 weeks during aerobic training improved their time to complete a 15-km cycling time trial by 3.4 min compared to 1.6 min in the placebo group ($p < 0.05$). Given these convincing results, the study of highly-trained

competitive female athletes training at a high volume and intensity was warranted. We expected these significant effects to persist in competitive collegiate athletes. However, we expected the magnitude of these effects to be somewhat less due to collegiate athletes' advanced training status, and thus a smaller margin of improvement in performance due to response of increased body iron stores. The goal of the proposed study was to determine whether marginal iron deficiency (IDNA) impairs the ability of moderately- to highly-trained female collegiate rowers to increase their training quality, as well as their performance in response to 6 weeks of iron supplementation, in addition to their usual endurance training.

The specific aims of the current study were:

1. To determine the prevalence of IDNA in a sample of female rowers at the beginning of a training season.
2. To determine how IDNA affects endurance training and performance at the beginning of a training season.
3. To determine how iron supplementation affects iron status, training and performance in IDNA female collegiate rowers.

It was hypothesized that:

- 1) IDNA is highly prevalent among female collegiate rowers.
- 2) IDNA affects endurance performance in female collegiate rowers both in and outside of the laboratory.

- 3) Iron supplementation of IDNA rowers will improve iron status, and consequently, training quality via increased energetic efficiency.
- 4) Iron supplementation of rowers with and without IDNA will prevent the deterioration in iron status resulting from endurance training, and thus significantly improve endurance capacity above the effect of training alone.
- 5) IDNA rowers with the most-compromised iron status will benefit the most from iron supplementation.
- 6) As training quality improves (with improved iron status), endurance performance will also improve; those with the most improvement in iron status will show the greatest improvement in performance.

Our long-term goal is to inform standard iron status screening and intervention practices among female collegiate endurance athletes in order to improve health status and benefit endurance athletic performance.

This study was conducted in three phases. Phase 1 was a cross-sectional study designed to describe the iron status of a diverse sample of female collegiate rowers around central New York state. Iron status was screened with a venous blood sample, and demographic and other health and self-reported performance data were also collected. One-hundred and sixty-five female collegiate endurance athletes were screened to identify IDNA subjects (sFer <20 µg/l, Hgb >12 g/dL) for an iron supplementation trial.

Phase 2 was a cross-sectional study designed to measure and compare the metabolic and functional consequences of ID in a sample of highly-trained female rowers across a broad range of both fitness levels (novice to varsity) and iron status (normal, ID, and IDNA). This cross-sectional study was an analysis of the baseline data for potential RCT participants (IDNA) at the beginning of a training season. In addition to those IDNA subjects participating in the supplementation trial, we included a sample of non-anemic, non-iron deficient rowers. These subjects completed all baseline protocols in the lab, and recorded one week of training activities, in addition to all other baseline data collected. This cross-sectional study enabled us to investigate potential relationships between iron status and early training season performance.

This plausibility analysis was useful, in light of the putative mechanisms (correlations between iron status and physical performance), to explain how iron status may affect physical performance. These analyses suggested relationships between iron status and performance, but did not provide strong causal evidence, as temporal relationships between iron status and performance cannot be determined in a cross-sectional study. We did, however, need to identify and control confounding factors related to both iron status and performance to control bias.

Phase 3 was a randomized, placebo-controlled supplementation trial designed to explore how IDNA and iron supplementation affect iron status, performance, and training over 6-weeks of rowing training. Rowers with normal iron status were included in this study to examine training effects (if any) on iron status and

performance. This study was designed to elucidate the cause-effect relationship(s) between iron status (and iron supplementation), training and performance.

This dissertation is structured in the following manner: after the literature review (*Chapter 2*), the general methods of the three studies are presented (*Chapter 3*). Research papers are then presented in *Chapters 4-8* in chronological order (*Chapters 4-5*, Cross-sectional studies; *Chapters 6-8*, results of RCT), followed by a general discussion (*Chapter 9*).

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CHAPTER 1

LITERATURE REVIEW

Overview: This chapter will review aspects of iron metabolism (absorption, storage, assessment of iron status). Dietary and supplemental iron is then discussed, followed by a discussion of how IDNA affects physical performance and endurance training.

Iron Absorption and Balance

Iron's low solubility rules out urinary or intestinal excretion as a means of maintaining iron balance, thus iron balance is regulated by three mechanisms: 1) continuous re-utilization of iron from catabolized erythrocytes (RBCs); 2) mobilization of iron stores; and 3) regulation of iron absorption from the intestines. Iron turnover is mediated by the destruction and recycling of RBCs by the reticuloendothelial system (1). Erythrocytes contain 80% of the body's functional iron and have a 120-day lifespan. Eighty-five percent of (non-storage) iron derived from hemoglobin (Hgb) degradation is re-released to the body as iron bound to transferrin or ferritin in the plasma, returning the iron to RBC synthesis in bone marrow and to other tissues; 0.66% of the body's total iron content is recycled this way each day (1). Uptake and distribution of body iron is regulated by the synthesis of transferrin receptors (sTfR) on the cell surface, also influenced by cellular iron status. sTfR is ultimately regulated by iron-response proteins (IRP-1, IRP-2) binding to mRNA iron-response elements (IREs) (1). As the cellular pool of iron decreases, there is up-

regulation of iron intake into cells and a down-regulation of the synthesis of iron-storage proteins. In the absence of iron, IRP-1 binds to the IREs of various iron proteins to regulate translation of mRNA transcripts (1).

Access to ferritin, the body's iron storage protein found mostly in the liver and spleen, is another mechanism by which iron balance is achieved. Ferritin binds iron in periods of relatively low need and releases it to meet excessive iron demands. Storage iron concentration varies from 0-15 mg/kg body weight, depending on age, sex, and individual iron status (1).

The negative feedback control on iron transport and absorption also regulates intestinal iron absorption: iron transport is optimized when an individual is iron depleted, and is limited when iron stores are replete. Hepcidin is a hormone (signaling protein) from liver cells that links with its receptor ferroportin to regulate iron release from macrophages and enterocytes (1). Prolonged negative iron balance due to either chronic basal losses or an acute rapid blood loss depletes iron stores, which can contain 2000-3000 mg of iron. Storage iron bound in ferritin is used to meet daily iron requirements not provided by dietary iron, and will become depleted with negative iron balance. Iron mobilized from tissue (stores of <5 mg) is transported by transferrin, which is 25-50% saturated with iron in iron-replete individuals (1).

Iron losses from body in female athletes

About half of the basal iron losses are from blood loss and occur primarily in the gastrointestinal tract. Both gastrointestinal losses and the menstrual iron losses are influenced by individual iron status; with a depletion of stores, menstrual and basal

iron losses will decrease. In a state of more severe iron deficiency, skin iron losses, which are generally minimal, may also decrease. Iron balance (state in which absorption equals losses) may be present not only in normal subjects, but also during iron deficiency and iron overload (2). As mentioned, iron balance is maintained by the regulation of iron absorption as it is related to iron loss. Iron is mainly lost with cells exfoliated from the skin and from the interior surfaces of the body (intestines, urinary tract, airways). The total amount lost is estimated at 1.4 mg per day (3), and is affected by iron status.

Menstruating women carry an increased risk for iron deficiency due to monthly menstrual blood loss (~34 ml/month), regardless of training status (4). Menstruation is the primary source of iron loss and this loss increases menstruating women's iron requirement by 0.55 mg/d (5); oral contraceptives can reduce this loss. Blood loss via donation contains 210-240 mg of iron per unit (pint) of blood donated.

Runners and other long-duration weight-bearing athletes may experience greater gastrointestinal iron losses through feces and hematuria than non-athletes (3, 6). Telford et al (7) found that in male triathletes, plasma free Hgb and sHaptoglobin were increased after exercise, and that this increase was four times greater after a run phase than a cycle phase, suggesting foot-strike is a major contributor to hemolysis during running activities.

Earlier studies have suggested that sweat iron losses could be considerable, especially in a hot, humid climate (3). However, Brune et al (8), conducted a study taking precautions to avoid the contamination of iron from the skin during body sweat collection and showed that sweat iron losses, though directly related to the volume of

sweat loss, were negligible (22.5 µg iron/L sweat), and unlikely to impact iron requirements (8). Waller and Haymes (9) found that the sweat rate of female athletes actually decreases over time and that the greatest concentration of iron in sweat occurred during the first 30 min of exercise. This loss was lower in a hot versus a neutral environment. Sweat iron concentration in this study was related to sFer, suggesting a possible conservation of iron with reduced stores. The researchers estimated that 5.7% of daily iron absorbed (1.2 mg/d) would be lost by female athletes during that first hour of exercise, contributing to a depletion of iron stores over time (9).

Strong evidence showing that prolonged training impacts iron stores is lacking. In a study using radioactively-labeled iron, Ehn et al (10) showed that the half-life of body iron in eight runners was ~1000 d, which was not statistically-significant, but shorter than 2100 and 1300 d in non-athletic males and females. No controls were used in this study, limiting the strength of the evidence. In another study, using a factorial analysis approach Brune et al estimated that athletes were excreting 3L/d of sweat containing 0.21 mg/L iron (which equals 0.6 mg iron/d lost in sweat) (8), which may also be a possible contributor to athletes' increased daily iron losses with prolonged training.

From a dietary intake perspective, many female athletes, attempt to lose weight or body fat in hopes of improving performance or meeting weight expectations of their specific sport. In their attempts to lose weight and fat, or gain muscle mass, some athletes resort to dietary energy restriction either seasonally or year-round, which can potentially be harmful to their performance and their health (11-13). As energy intake

decreases, dietary iron consumption is likely to be compromised. Dietary energy restriction is especially prevalent in female athletes involved in weight-sensitive sports such as figure skating, gymnastics, diving, sprinting and long-distance running, rowing, and swimming, though dietary intake is rarely evaluated with precision in these populations (14-17). Studies examining dietary intake (via 7d records, not including supplements) of endurance athletes have reported average iron intakes ranging from 9 – 42 mg of iron per day (18, 19). Steen et al (20) found that although a sample female rowers met the RDA for kcal and many vitamins, they had suboptimal intakes of many minerals, including iron. The rowers in this study reported low intakes of sources of bio-available iron, such as beef and poultry.

Iron requirements

The recommended daily allowance (RDA, meets the needs of almost all individuals in a group) of iron for pre-menopausal women is 18 mg of iron/day. A tolerable upper level (TUL, maximum level likely to pose no risk of adverse effects) has been set at 45 mg/day (21). The estimated average requirement (EAR, used to calculate RDA) is 8.1 mg/d. Proposed mechanisms for increased iron requirements in female athletes include the losses discussed above, however, it is hard to conclude how exercise affects iron requirements in female athletes based on available research.

The U.S. military, however, has set iron's RDA for female soldiers who are training at 22 mg/day (22). Estimates of basal losses (1.7 mg/d) by Weaver and Rajaram (23) were used to base these recommendations on iron requirements for training military personnel, accounting for sweat losses and training duration/ work

load. This results in an EAR of 13 mg/d. The EAR, along with the SD for requirements (4.66 mg/d) was then used to calculate an RDA for training female soldiers ($RDA = EAR + 2 SD$) of 22 mg/d (22).

If female athletes (including military personnel and other active females) do require more iron, it may most likely be for those women engaged in weight-bearing activities which result in gastrointestinal losses and foot-strike hemolysis. Few study protocols control for dietary intake or menstrual status, or have standardized testing and treatment protocols or measures and classification of iron status (markers and cutoff points). Screening for ID and monitoring iron status in athletes (and soldiers) upon recruitment, during training, and throughout the competitive season (and military deployment) may help to substantiate the argument over increased iron requirements for female athletes (and soldiers).

Bioavailability of Dietary Iron

Dietary iron is absorbed in the small intestine where it is taken in by and transferred across mucosal cells into the blood. There are specific receptors on the brush border for various forms of iron and its transport protein within the mucosal cells for moving iron into plasma (1). Dietary iron is classified as heme or non-heme iron, and the bioavailability of these two forms is different. Heme iron is soluble in an alkaline environment and is absorbed into the intestinal mucosal cells as intact metalloporphyrin molecules, so its absorption is not affected by dietary factors other than calcium. Primary sources of heme iron are animal tissue, which contains hemoglobin and myoglobin (see Table 2.1). The average absorption of heme iron

from meat-containing meals is about 25% (24) , which can vary depending on iron status (from ~ 10% during iron repletion to ~ 40% during iron deficiency to (25) , and on dietary calcium, which negatively influences the absorption of heme iron (26).

Non-heme iron is the main form of dietary iron and is obtained from cereals, legumes, fruits, and vegetables (see Table 2.1). Non-heme iron has variable solubility depending on the amounts of ferric and ferrous iron, as well as the amount of dietary inhibitors (phytates, phenols, calcium) and enhancers (vitamin C, animal tissue) of absorption. These compounds form large polymers or precipitate with the non-heme iron making it inaccessible to the mucosal cells, thus reducing iron absorption.

Table 2. 1. Iron content of selected iron-rich foods – USDA National Nutrient Data Base

Food	Serving Size	Mg Iron/Serving
Ground beef	4 oz, cooked	3
Roast beef	4 oz, cooked	35
Chicken, dark meat	4 oz, cooked	3
Tuna, fish	3 oz, cooked	1
Ham	4 oz, cooked	2
Lentils	1c, cooked	7
Chick peas	1 c, cooked	5
Eggs	2, cooked	2
Nuts/seeds	¼ c	1
Bagel	1-3.5 inch	4
Cereal, fortified	1 c	5 (varies)
Raisins, dates	½ c	1
Spinach	1c, boiled, drained	6

Iron supplementation

Ferric iron's absorption rate is about one-fourth of ferrous iron, and ferric iron supplementation was not effective in improving iron status in IDA patients (27). The more bio-available ferrous iron is available as different salts: sulfate, lactate, fumarate, glutamate, and gluconate, all of which have comparable rates of absorption and incidence of gastrointestinal side-effects (e.g. constipation, nausea, vomiting, diarrhea, darkened stools) (3). Twelve to 13% of an iron supplement is elemental iron,

available for absorption in the body. Average absorption from ferrous salts is 20% from sulfate, 33% fumarate, and 12% gluconate (28).

Supplementation of more than 45 mg/d elemental iron (e.g. as 225 mg ferrous sulfate) increases chances for gastrointestinal side-effects, which are still possible with lower-dose iron supplementation, but these effects may not be experienced in all subjects (3). Side effects can be minimized by using a slow-release preparation (slow-Fe), which reduces the concentration of iron on the mucosal surface, though these slow-Fe preparations are more slowly absorbed than quick-dissolving formulations. Iron supplements containing heme iron have a higher bioavailability and lower incidence of side effects than those containing only non-heme iron (29, 30). Administering ferrous salts immediately after a meal decreases absorption (3). Since iron from non-heme iron supplements enters the same non-heme iron pool in the intestinal lumen as does the iron in food, the dietary factors affecting bioavailability of dietary non-heme iron will also affect the absorption of supplemental iron.

Data from a recent analysis of NHANES 2003-2006 show that 53% of US females were consuming dietary supplements. Fifteen percent of supplement-consuming females aged 19-30 y reported taking one containing iron (31). Data from NHANES III showed that 23% of pre-menopausal women were consuming iron-containing supplements (32), and 40.7% of supplement-users were consuming more than the RDA for iron (≤ 18 mg/d) from supplements alone (33). Additionally, no difference in iron status between supplement-users and non-users was shown in this study (33). Survey data report that 7-30% of female athletes consume an iron

supplement (34-36), although a larger number of female athletes (72.7%) report consuming a multiple vitamin-mineral supplement that contains iron (36) .

Prevalence of Iron Deficiency

Iron deficiency (ID) is the most common micronutrient deficiency in the United States. There is a wide distribution of Hgb concentration in healthy, non-ID subjects (in women, 120–160 g/l; in men, 140–180 g/l) (37). While 4% of US women 20-49 years of age are iron deficient anemic (IDA), the prevalence of anemia underestimates that of ID in the population by more than 50% (38), as more than 12% are iron deficient without anemia (IDNA) based on sFer<12.0 (or 20.0) $\mu\text{g/L}$ (39, 40). Pre-menopausal women are at increased risk of ID due to the additional iron losses via menstruation. IDNA is particularly prevalent among college-aged women, female athletes and soldiers and the physically-active (41, 42). These women are at a higher risk of IDNA due to their particularly prevalent low energy intakes (and consequently low iron intakes), as well as their training status, which may increase basal losses. Our research group has found the prevalence of IDNA to be about 30-35% in a sample of female collegiate endurance athletes (43). As of 2005, nearly 165,000 female athletes were competing in NCAA sports, thus many active college women are affected by IDNA and would benefit by ameliorating the problem (44).

Assessment of Iron Status

The most common consequence of iron deficiency is anemia, or blood hemoglobin (Hgb) concentration below a specified level (12 g/dL for women of child-

bearing age). The main use of the cut-off value in defining anemia is to compare prevalence between population groups. During the development of a negative iron balance in subjects with depleted iron stores (serum ferritin concentration $<12 \mu\text{g/L}$), there will be a decrease in Hgb, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV). This will lead to an overlap in the distributions of Hgb in iron-deficient and iron-replete women. The extent of overlap depends on the prevalence and severity of iron deficiency (37).

Anemic young adult females are commonly identified using $\text{Hgb} < 12 \text{ g/dL}$ as the clinical definition of anemia. Hgb concentration decreases with a decline in hematopoiesis, although this does not only occur as a result of depletion of iron stores. In the early stages of endurance training, hematological adaption can be observed in athletes. For example, although absolute Hgb mass is increased with training due to erythrocytosis, there is often a dilution of Hgb, Hct, and RBC (termed *psuedo-* or *sports anemia*) with increased plasma volume associated with endurance training due to exercise-induced release of aldosterone, renin, and vasopressin, as well as increased synthesis and retention of plasma albumin (45). This is a transient condition common in endurance-type athletes (runners, swimmers, rowers). This rapid increase in plasma volume is proportional to the amount and intensity of training (from 5-20%), and disappears within a few days of adaptation to a training load (or as one becomes de-trained).

Hemolysis induced by exercise and resulting in low blood Hgb, hematuria, and hemoglobinuria has also been proposed as a mechanism of increased iron loss in athletes, particularly in runners (7). The increases in RBC mass and decreases in

haptoglobin with hemolysis, have been attributed to increased turnover and destruction of red blood cells via inevitable mechanical trauma during various types of endurance training (e.g. “foot-strike hemolysis”; muscle contraction in capillaries; increased renal blood flow and blood pressure) (46, 47).

Iron deficiency anemia (IDA) occurs when iron stores become depleted (sFer<12 µg/L) and Hgb falls below 12 g/dL. Consequently, insufficient amounts of iron will be delivered to transferrin (Tf, iron transport protein), and the binding sites for iron on transferrin will contain less iron, resulting in a reduction in transferrin saturation (TS), and an increase in total iron binding capacity (TIBC), and ultimately reduction in iron delivery to cells and tissues in need, as well as serum iron. When TS drops to a certain critical level, RBC precursors will not have enough iron to form Hgb. As liver cells receive less iron, more Tf will be synthesized, and the concentration of Tf in plasma will increase. The uptake of iron is related both to TS and the number of transferrin receptors (TfR) on the cell surface (1). There is a marked diurnal variation in the saturation of Tf because of the high turnover rate of iron in plasma, making it difficult to evaluate iron status from a single measurement of TS.

To identify individuals who are iron deficient without anemia (*IDNA*), iron status can be examined using a number of indicators (or combinations of indicators) to elucidate various body iron storage pools that are useful for understanding the functional consequences of ID. With excessive iron loss or inadequate iron intake, body iron stores decline and ID occurs. When iron stores have become completely

depleted but Hgb has not yet declined to a level indicative of anemia, this is a state of *IDNA*.

The best single indicator of iron stores is serum ferritin (sFer), which is stored predominantly in the liver, spleen, and bone marrow. Each $\mu\text{g/L}$ ferritin in the plasma represents about 8 mg of storage iron (1). There is also an inverse relationship between ferritin and iron absorption, indicating that sFer is a sensitive indicator of body iron stores. sFer identifies iron depletion at concentrations as high as $20.0 \mu\text{g/L}$, though the clinical cut-off for iron deficiency is $12.0 \mu\text{g/L}$ (1, 3). A sFer $<12.0 \mu\text{g/L}$ indicates complete depletion of Fe stores in the bone marrow; $12-20 \mu\text{g/L}$ indicates minimal stores; and $>20.0 \mu\text{g/L}$ indicates adequate Fe stores. Based on NHANES III, median sFer was $36-40 \mu\text{g/L}$ for healthy, non-pregnant menstruating women of child-bearing age (21). Normal or high ferritin levels, however, do not guarantee adequate iron stores. Ferritin is an acute phase protein (explained below) and may therefore vary in certain conditions without changes in iron storage, such as infection, inflammation, and increased training intensity which may mask potential iron depletion (48, 49).

Serum transferrin receptor (sTfR) is an index of functional iron deficiency, and is independent of sex, inflammation, and training status. Transferrin (Tf) distributes iron to cells that have transferrin receptors (TfR) on their cellular surface. Almost all cells have TfR on the cell membrane, with the largest number of TfRs found in rapidly-dividing cells, Hgb-synthesizing tissues, and the placenta (50). As mentioned previously, a negative feedback loop controls TfR synthesis: low iron stores results in stimulation and excess iron suppresses TfR synthesis. sTfR provides a continuous measure of iron status in an individual, rather than using categorical classification.

sTfR, independent of Hgb, is directly correlated with total mass of erythroid precursors and increases in proportion to severity of ID, and is therefore indicative of IDNA. In ID, as there is increased demand for iron at the level of skeletal muscle tissue, increased sTfR (>8.0 or 8.5 mg/L, depending on the assay kit used) indicates that this demand is not being met by circulating iron. sTfR identifies IDNA individuals who will potentially benefit from iron supplementation (51). As sTfR reflects overall erythropoiesis, endurance athletes should have higher sTfR concentration levels than untrained or in moderately-trained individuals (52, 53). Banfi et al (54) found that the sTfR of female rugby players increased over 10 months of training and during competition.

Mean corpuscular volume (MCV) is one of the last parameters to change with onset of iron deficient erythropoiesis. Ferritin and MCV models use multiple criteria to determine IDA vs. IDNA vs. anemic, non-ID vs. non-ID, non-anemic. If an individual has more than two abnormal values (sFer, FEP, MCV, TS), they are considered ID.

In 1986, Cook et al developed an algorithm to estimate body iron (55) that relies on multiple criteria of iron status (Hgb, sFer, FEP, TS) and several equations to estimate body iron stores and distinguish ID from non-ID. In anemic individuals, Hgb drives the model, and in IDNA, sFer drives the model. Mild (though functional) ID may go undetected when using only Hgb and sFer, as individuals with Hgb in the upper-normal range must lose **20-30%** of body iron before ID can be detected by anemia (48). Recently, Cook et al (56) have developed another algorithm to estimate

total body iron (TBI) from the log of sTfR/sFer, which permits detection of mild tissue ID in non-anemic individuals:

$$\text{Iron stores (mg/kg)} = -[\log(\text{sTfR} * 1000 / \text{sFer}) - 2.8229] / 0.1207$$

Inflammation and the assessment of iron stores

Inflammatory processes present a challenge when evaluating measures of iron status. Although sFer is the most common index of iron stores, and reflects iron stored in the liver, it is an acute-phase protein (APP) and can be elevated in an inflammatory state (e.g. infection, post-exercise), potentially masking ID (53, 57, 58). The acute phase response (APR) is a protective inflammatory response. Following an injury (e.g. muscle tear) or acute or chronic inflammation (e.g. allergic response or upper respiratory infection), both iron transport and absorption are suppressed. During this APR, ferritin levels become inflated by ~20-38% for several days, making accurate assessment of iron stores difficult, and possibly masking iron deficiency (59, 60). Iron is sequestered into ferritin, compromising iron availability for incorporation into Hgb. Therefore, it is important to estimate how much influence the inflammatory response may be having on iron status and its assessment.

An inflammatory marker such as C-reactive protein (CRP) or alpha-1-acid glycoprotein (AGP) can partially rule-out falsely-elevated sFer (60-62). These APPs have different temporal responses to inflammation. The peak response of AGP (5-6 d) matches that of ferritin (up to 10 d) more closely than does CRP (1-2 d). Rocker et al (52) examined the iron status of 12 female triathletes before and after a single competition (1.5 K swim, 20 K cycle, 10 K run) and found that sFer remained elevated

after correcting for hemoconcentration; no inflammatory markers were measured in this study. Beard et al (61) measured CRP and AGP along with sFer and sTfR in several studies to determine which APP performed better in predicting sFer levels responding to current inflammation. They found that only sFer was related to APP concentrations, though it had a poor positive predictive value (<72%) due to a low prevalence of inflammation in their sample. AGP, however seemed to be a better predictor of sFer. Most recently, Thurnham et al reported that correcting ferritin values for inflammation (using AGP and/or CRP) improved the assessment of iron status using ferritin, enabling correct classification of subjects as iron deficient (decreased number of false negatives) (60), however, an appropriate correction factor in training athletes has not been published.

Aside from the effects of the APR on sFer as an indicator of iron status, recent research has suggested that the iron regulatory protein hepcidin is the mediator between the inflammatory response and poor iron status via iron absorption (63). Endurance exercise has been shown to evoke an APR in post-exercise cytokines, and a 2-3-fold increase in pro-inflammatory and in inflammation-responsive (IL6) cytokines have been reported (64). These exercise-induced elevations may up-regulate hepcidin, which then enters the circulation to negatively control the export of iron from the intestinal enterocyte. Iron uptake by macrophages in response to hemolysis would not be retainable due to the internalization of the macrophage surface ferroportin channels (64). Thus, acute increases in hepcidin may lead to a decrease in the absorption of dietary iron (65). This absorption of iron from post-exercise meals may then be limited for a prolonged time period as a result of the effect on duodenal enterocytes

within the gut. Roecker et al (66) found that urinary levels of hepcidin 24-h post-marathon were 2.5 times greater than pre-marathon levels. The researchers suggest a possible exercise-induced increase in inflammatory up-regulation of hepcidin, which may lead to ID over time.

Several studies have shown associations between or increases in urinary hepcidin and inflammation post-exercise, suggesting that high levels of training may negatively affect iron status (46, 59, 64, 66-68). Most recently, researchers found that although serum hepcidin was not affected by basic combat training in female military soldiers, hepcidin was lower in IDA soldiers and was positively associated with sFer and CRP levels before and after training (69). This mechanism may explain the increased prevalence of iron deficiency in female athletes and/or changes in iron status with training, as well as the reduced potential for improved sFer with iron supplementation in athletes.

Relationship between iron status, physical performance, and physical activity

It is commonly known that anemia, the most severe form of ID, has severe consequences on physical performance due to the inadequacy of Hgb to transport O₂ to the peripheral tissues (70, 71). In an extensive review of the literature, Haas and Brownlie cited strong evidence to support the finding that IDA impairs aerobic capacity due to inability of Hgb to transport O₂ (72). However, iron plays an important role in muscle metabolism beyond O₂ transport (Figure 2.1).

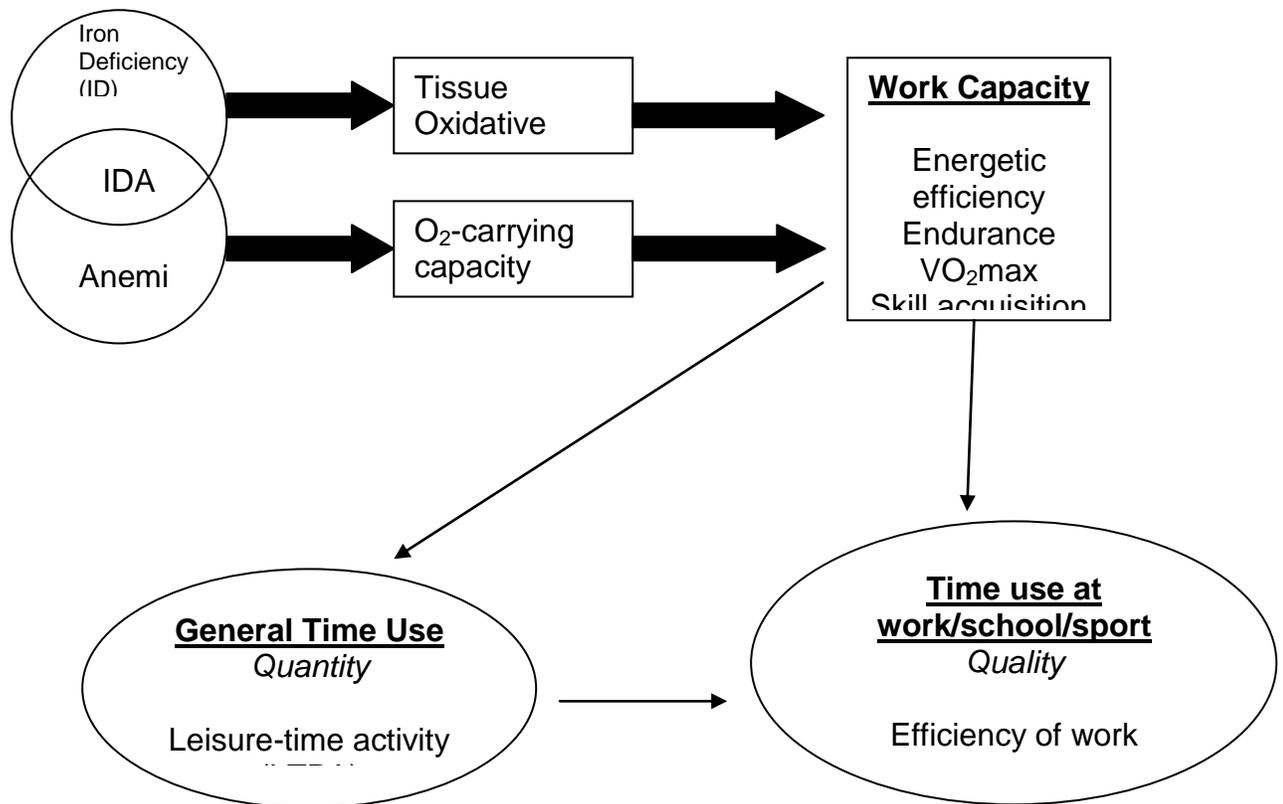


Figure 2.1. Effect of Iron deficiency on physical activity. Adapted from Haas and Brownlie (73).

There are many iron-containing enzymes involved in transforming chemical energy to mechanical energy (work) that are affected by iron depletion (energy produced by mitochondrial cytochromes, Table 2.2). About 5% of body iron is found in iron-containing enzymes, and 10% is found in myoglobin (muscle storage of O₂). These iron-containing enzymes are employed in oxidative metabolism in the transformation of chemical to mechanical energy via the Krebs cycle (TCA cycle through iron-sulfur proteins) and electron transport chain (ETC, through heme-dependent cytochromes).

Table 2.2. Iron-dependent enzymes

Heme Compounds	Non-heme Compounds	Iron-Dependent Enzymes
Myoglobin	NADH dehydrogenase	Lipid peroxidase
Cytochromes	Succinate dehydrogenase	Proline hydroxylase
Catalase	Xanthine oxidase	Lysine hydroxylase
Peroxidases	Aldehyde oxidase	
	Alpha-glycero-phosphate oxidase	
	Phenylalanine hydroxylase	

Evidence from human studies (70, 74, 75) supports findings from animal studies (76-82) that both mitochondrial content, as well as the activities of iron-containing oxidative enzymes in skeletal muscle mitochondria essential to O₂ utilization within working muscles are decreased in ID (independent of anemia), leading to reduced work performed and endurance capacity. Changes in these enzymes due to ID can occur in many tissue types, but it is the changes in skeletal muscle fibers that are most important relative to physical performance (77, 79).

Another consequence of IDNA related to physical performance is energetic efficiency (EF), which is defined as the ratio of the amount of physical work produced from a process (W) to the amount of metabolic work/energy that went into the process (energy expended, VO₂). Studies of IDNA non-athletes have shown that repletion of iron stores improves EF (83-85). This means that women with sufficient iron stores

completed the same exercise/workload at a lower energy cost than women with depleted iron stores. Iron is presumed to play a role in EF via oxidative capacity, or controlled in part by levels of iron-containing enzymes (e.g. muscle pyruvate oxidase) at the tissue level, as mentioned previously (77). A limitation of EF as an outcome is that the total amount of work done during exercise is the total sum of both internal and external work outputs, however, internal work (for example, the O₂ cost of breathing) usually goes unmeasured. Because EF is based on whole body VO₂ and is not a specific measure of muscle EF, this outcome is often corrected to isolate the EF of working muscle by subtracting baseline energy expenditure from total energy expenditure (termed net efficiency).

Lactate concentration is used as a proxy for anaerobic metabolism, and is highly correlated with endurance performance (86-90). Results from animal studies suggest that increased blood lactate is due to a reduction in enzyme activity involved in glycolytic pathways (76). Higher blood lactate concentrations during moderate exercise may increase one's susceptibility to fatigue and result in poor endurance performance – this has been shown in both animals and humans with ID. This means that those with ID are using more energetically- “costly” anaerobic energy pathways to produce the same amount of work compared to those with normal iron status.

Other functional consequences of ID found in both male and female athletes and non-athletes are shown in Figure 2.2. From a broader public health perspective, social and economic consequences of ID include low work/school productivity, increased food/energy needs, reduced time spent in leisure-time physical activities, and low-educability (55, 91), all of which apply to student athletes.

IDNA and Physical Performance in Non-Athletes

Our lab has completed a series of studies reporting the effects of iron deficiency on physical performance in untrained, ***IDNA*** women with differing experience in aerobic training (83, 92, 93). In the first study (n=30), Zhu and Haas (83) reported that the effect of iron supplementation in non-exercising women was mediated by changes in iron status (sFer), and it was concluded that IDNA reduces the efficiency with which work is performed at moderate levels of exertion. In the second study (n=42), Hinton et al (93) found that those supplemented with iron for 6 weeks while participating in an aerobic training program improved their time to complete a 15-km time trial by 3.4 min compared to 1.6 min in the placebo group ($p < 0.05$, see *Figure 2.3*). IDNA untrained women in our studies have also shown significant improvements in energetic efficiency (83, 84, 92, 94); resistance to local muscle fatigue (95); and maximal aerobic power post-training (94). Given the magnitude of these effects, the study of highly-trained competitive female athletes training at a high volume and intensity seems warranted. We expected to observe similar effects in trained athletes, although we predicted that the magnitude of the effect to be somewhat less, due to athletes' advanced training status, and thus a smaller margin of improvement in performance with improved iron status.

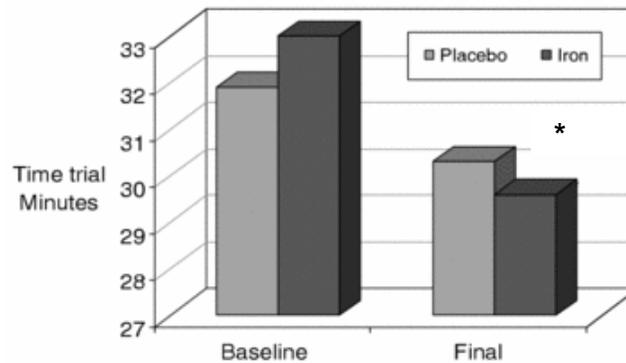


Figure 2.3. Improvements in time (min) for moderately-trained women to complete a 15-km time trial after 4 wk aerobic training while supplemented with either iron or placebo (* $p < 0.05$ iron compared to placebo, controlling for Baseline) (93).

IDNA and Physical Performance in Athletes

Studies of how iron status affects non-anemic endurance athletes are limited, but research suggests that female endurance athletes and military soldiers with IDNA have impaired physical performance (47, 85, 96-98). Friedmann et al (96) studied 40 young male and female IDNA athletes and showed that those athletes who were supplemented with iron for 12 wk showed a 10% improvement in endurance ($p < 0.001$), which was also significantly different from the placebo group ($p < 0.05$). Rowland et al (74) also reported improvement in endurance time in IDNA female runners after supplementation, although the placebo group in this study inexplicably decreased their endurance time. Other studies have shown decreased blood lactate, as well as improvement of (mildly anemic) Hgb status in non-anemic athletes supplemented with iron (87, 99). Most recently, Hinton et al found that after 6 weeks of iron supplementation, recreational athletes' ($n = 3M, 17 F$) post-trial energetic efficiency was significantly increased (+1.1%) compared to placebo (+0.7%), although the post-trial EF measure was not significantly different between the treatment groups

(85). A recent study of female military soldiers with depleted iron status showed improvements in 2-mile run time after basic combat training (100). These studies did not investigate the role of training itself, and how IDNA may affect the intensity of training in order to affect post-training performance. Studies examining the effect of IDNA on training in trained individuals have not been conducted.

IDNA and Physical Activity in Non-Athletes

While much of the focus has been on the relationship between IDNA and physical performance or work capacity, only a few studies have attempted to address the relationship between IDNA and training or other leisure-time physical activities (LTPA). After inducing ID in animals, voluntary physical activity decreased in a dose-response manner (increased severity of ID, further decreases in physical activity) (101-103).

Human studies conducted in field settings have shown that women with iron deficiency with and without anemia working on tea plantations (104) and in cotton mills (105) significantly increased their voluntary physical activity when provided with iron supplementation. Ameliorating IDNA also contributes to improved worker productivity (72, 106-108) and quality of life (72, 109, 110) at both the macro (economy) and micro (individual, family) levels. To the best of our knowledge, there have been no published studies examining IDNA's effects on LTPA in athletes who are in-training. We do not know how athletes who are IDNA compensate for the effects of IDNA outside of their sport training by, for example, reducing their time spent in activities at home, school, and work.

Training intensity and load

Endurance athletes at the collegiate level spend many hours per week participating in specific training for their sports in order to improve their competitive performance. Training can be quantified in terms of frequency, intensity, time (duration), and type (of activity), commonly referred to as “FITT” (111). The intensity of athletes’ training is the major variable influencing the overall effects of training on endurance performance (112). Intensity is a function primarily of speed (miles per hour, mph; strokes per minute, spm, etc) for endurance athletes, because they are focused on completing a certain distance in as little time as possible.

A basic concept of training is that of progressive overload, or of the adaptation to increased amounts of work over time. Training load is a combination of intensity, duration and frequency. To maximize athletes’ potential benefits of or adaptation to training, the level of the training load must be appropriate for both the individual and for the outcome (e.g. endurance performance in a given event) (112). Specificity is also important, as adaptations to training should result in training of the muscles required to perform the activity, as opposed to general aerobic or strength conditioning (113). Specificity also becomes important when deciding upon the mode of testing to measure the effects of training (or other variables) on sport-specific performance (114, 115).

The majority of pre-season endurance training programs consist of an initial conditioning phase lasting 4-6 weeks, encompassing a high volume of general aerobic conditioning (cross-training) and strength training. During this period, much of training is done at or just below an oxygen consumption above which aerobic energy

production is supplemented by anaerobic mechanisms (around 75-85% VO₂ max). This training level is known as the anaerobic threshold (AT), and causes a sustained increase in lactate and metabolic acidosis (116). Training then becomes preparation for competition, incorporating technical skill of the sport (speed, coordination, etc). As the competition approaches, training focuses on sport-specific high-intensity, short duration activities with recovery time. Maximal oxygen consumption (VO₂ max) can be maintained up to 28d with a decrease in training of about 30% (maintenance of 70-80% of maximal training load). If a training load volume is not maintained, VO₂max can decrease 4-14% within 4 weeks (111).

There are many changes to the anaerobic and aerobic energetic systems with endurance training. Anaerobic changes include increased levels of anaerobic substrates (ATP, PCr, free creatine, glycogen); increased number and activity of enzymes that control the anaerobic phase of glucose catabolism; and increased capacity to generate high levels of blood lactate during exercise (via increased levels of glycogen and glycolytic enzymes, improved pain/fatigue tolerance) (111, 117).

Endurance training also induces several adaptations in a variety of functional capacities related to O₂ use and transport within skeletal muscle, namely increases oxidative capacity, where iron plays an essential role. Endurance-trained muscles contain larger and more numerous (2x more) mitochondria (site of O₂ diffusion, oxidation of ACoA → NADH, FADH₂, and electron transfer of O₂ → NADH, FADH₂) than less active muscle fibers. This increases mitochondrial capacity (via increased aerobic system enzymes, including those dependent on iron) to aerobically generate ATP (111, 118).

This increased enzymatic activity increases an endurance athlete's ability to sustain longer duration, higher-intensity activity without significant blood lactate accumulation (118, 119). High blood lactate levels are an indication that the glycolytic anaerobic pathway has been used during physical work, and serves as a reflection of the balance between lactate production and its removal. As mentioned, high lactate concentrations during exercise may result in increased fatigue, poor endurance performance, and/or sub-optimal maximal exercise performance (VO_2max).

Endurance training increases an athlete's capacity to mobilize, deliver, and oxidize fatty acids for fuel during sub-maximal exercise, which spares carbohydrate for maximal exercise. Increased fat catabolism is due to increased blood flow within trained muscle; increased numbers of fat-mobilizing and –metabolizing enzymes; improved muscle mitochondrial respiratory capacity; and decreased catecholamine release at a given level of work (111, 117, 119).

Trained muscle also has a greater capacity to oxidize carbohydrate during maximal exercise, as glycogen is spared with increased fat oxidation. Additionally, endurance athletes have larger slow-twitch (ST) muscle fibers than fast-twitch (FT) fibers within the same muscle; ST fibers have a high capacity to aerobically produce ATP and contain large amounts of myoglobin (111). Work efficiency plays a significant role in endurance exercise performance. The O_2 cost (ml/kg/min) of an activity at a given workload or power output (W) varies with individuals' fitness levels, as well as technical/skill level (biomechanical factors). The efficiency with which ATP is converted to work depends upon muscle fiber type and mitochondrial density (117).

There are also a number of specific cardiovascular adaptations to endurance training which augment O₂ delivery to active muscle. The heart's mass and volume increase with long-term aerobic training, just as skeletal muscle mass increases with progressive overload. Athlete's plasma volume increases from 5-20%, without changes in RBC mass, as soon as within 24h of the first training session, and persists as long as adaptation to progressive overload continues (45, 120). This increased plasma volume enhances circulatory reserve, increases stroke volume and O₂ transport. Resting heart rate (HR) and sub-maximal HR are also decreased (by 12-15 bpm) in the endurance-trained athletes, and is a main indicator of training improvement, reflecting increased stroke volume and cardiac output (111). Blood flow to active muscle improves with training, which increased muscles' ability to deliver, extract and use O₂. This is due to increased cardiac output and enlargement of capillary arteries and veins, as well as increased capillarization per gram of muscle (111).

It has been suggested that the iron status of experimental animals and humans is related to their physical training, but the direction of this relationship is unclear (49, 80, 81, 103, 121-123). Many studies have examined changes in iron status that occur with training, but the effects of iron status on *training itself* (training intensity, time) have not been adequately evaluated. Ashenden et al (49) examined the effect of seasonal variation in hematological parameters related to the onset of competitive sports training in female athletes, including rowers and court-sport athletes, and found a 25% decline ($p < 0.01$) in mean serum ferritin concentrations for all athletes during the training season, although physical performance measures,

training intensity and volume, and sTfR were not evaluated. Schumacher et al (47) screened 747 male athletes and found that increased physical duration and work load (self-reported training) was associated with decreased sFer. Similar results were found by Petersen (124) who found that after examining 18 swimmers over a 16-week training season, sFer had decreased by ~30%. More recently, McClung et al studied female soldiers before and after basic combat training and found declines in iron status (decreased sFer, increased sTfR) after 9 weeks (125). None of these studies assessed inflammation or controlled for changes in plasma volume. Nor did these studies measure dietary intake, or use standardized measures of training or performance. Furthermore, none of the mentioned studies have separated conditioning phase training periods from pre-competition phase/steady state periods.

Rowing

Rowing is among the oldest modern organized sport (126). Rowing, as a weight-supported sport, requires a high level of coordination and sophisticated motor control, as rowers need to coordinate their body movement with the oar's movement, as well as maintain boat balance. Biomechanically, rowers need to expend more power (W) to overcome higher drag resistance in order to increase boat speed. Power from rowers' legs contribute to about 50% of rowing power; trunk muscles contribute about 30%, and arms 20-25% (126). Mechanical efficiency of rowing at <25 spm is 18% and increases to 20-23% at 35 spm in experienced rowers; Novice rowers are 10% less efficient. The metabolic cost of a race assumes constant mechanical

efficiency of 22%, which is equivalent to about 5.3 L O₂/min for elite female rowers (126, 127).

Specific traits associated with competitive success in rowing include tall stature and high muscle mass, which yield a greater potential leverage and high power output (126, 128). On average, successful or elite female rowers are about 182 cm tall and weigh 80 kg, and 15-25% body fat (126). Anthropometrics and body composition are two important predictors of rowing performance. A tall height contributes to a higher body mass and strength endurance, and increased muscle mass contributes to both strength and VO₂ (r=0.73); aerobic power has been shown to increase as a cubic function of stature (126, 129). Rowers have a high proportion of active muscle mass to propel the dead weight of the boat, oars, and coxswain (if rowing in a crew). About 75% of rowers' total muscle mass is used during rowing, which is relatively high compared to weight-bearing sports due to the fact that rowers' mass is supported in the boat (126).

Rowing ergometers (aka "erg") have been used as rowing training and valid testing devices since the 1960s. A standard rowing ergometer (Model C, Concept 2, Morrisville, VT, USA, Figure 2.3) is used by rowers of all levels of skill and competition, especially during the winter/indoor training season. Rowing ergometer "scores" are used regularly by coaches to assess performance and assign boat placement/racing line-up throughout both Fall and Spring rowing seasons. Collegiate coaches routinely use the 2K erg score, as well as the maximal aerobic power (MAP) test to assess rowers' training progress. A standard ergometer is fixed to the floor, and all of the rower's power output goes into an air-braked flywheel, which simulates drag

through the water. Power generated by the air friction on this flywheel is measured and converted into a virtual boat speed by a small computer monitor, using the cubic relationship between power and velocity (PM2+, Concept 2, USA; $W = 2.8/\text{pace per } 500 \text{ m}^3$).



Figure 2.3. Concept 2 Rowing ergometer and output monitor, <http://www.concept2.com>

A caveat to the use of the ergometer is that it does not require the same amount of teamwork and coordination or technical and mechanical skill as does the boat and oars on the water, although studies have shown good correlation between ergometer and on-water performance. Urhausen (130) compared physiological responses during rowing on an ergometer and in a single scull on the water in 17 male rowers and found no significant difference between the 2 modes of rowing. Mikulic (131) found that a 2K rowing ergometer test was significantly correlated ($p < 0.05$) with self-reported competition boat performance a sample of 398 rowers, the strongest correlations being for ergometer performance times in single sculls ($r = 0.92$; $p < 0.001$) versus larger boats (quads, fours, and eights, $r = 0.31-0.70$; $p \leq 0.039$). Simulated races (5-6K, 2K)

are commonly used as testing protocols on the ergometer (129), and have been shown to represent final minute maximal VO_2 values in the laboratory (132).

Rowing training consists of about 75-85% aerobic work (128), which increases aerobic capacity at the cellular level (mitochondrial changes, capillary density, enzyme changes), where it is presumed iron plays an important role, as discussed previously. Row-specific training is high-intensity (intervals of up to 95% $\text{VO}_{2\text{max}}$), long duration (bouts of 30-60 min), and high frequency. Rowers do the majority of their training at or above their anaerobic threshold (around 75-85% $\text{VO}_{2\text{max}}$). Rowing-specific training (in boat or on ergometer) yields significant effects on rowing performance (technical skill, efficiency), as this specific training works the muscles and transport systems that support the action of rowing (128, 133). During the transition from off- to in-season, elite rowers can increase their $\text{VO}_{2\text{max}}$ by about 10% (5-15 ml $\text{O}_2/\text{kg}/\text{min}$) (126). As high-intensity endurance athletes, rowers have many more and larger slow twitch (ST) muscle fibers than fast twitch (FT) fibers, leading to their high aerobic capacity and anaerobic thresholds (86, 128). ST fibers have 50% higher oxidative capacity, and 50% lower glycolytic capacity than FT fibers; in short, ST rely more on fat as an energy substrate and are the first to be activated during endurance activities, such as rowing.

Due to rowers' variable seated body positioning in the boat or on the ergometer, they have increased ventilation (V_E , increased breathing frequency and lower tidal volume) than other endurance athletes, and employ a cyclic breathing pattern to compensate for this (134). During rowing, $V_E: \text{VO}_2$ equals or exceeds that of most endurance athletes. Lungs of rowers reflect their larger body size/height (vs.

runners) (126, 134). Rowers' exhalations and inhalations are performed in phase with rowing strokes (2 breaths: 1 stroke). Cardiac output and stroke volume are also affected by rowers' body positioning. Central blood volume is reduced following rowing due to increased perfusion pressure and muscle vasodilation. There is also a short diastolic interval (filling of the heart with blood) during rowing, and venous return to the heart is enhanced (126).

Rowers' pacing pattern is also important to endurance performance. At the beginning of a 2K race, there is a brief sprint (30-40 s, high spm), resulting in a high O_2 deficit (anaerobic) early in the race. For the next 4-4.5 min, the work is aerobic (reduced spm), followed by a final sprint (anaerobic) to the finish during the last minute of the race. Thus, rowers are performing near maximal work capacity for the duration of an entire 2K race (128, 133). VO_{2max} is a very important determinant of rowing performance. The aerobic contribution of metabolism to power output has been reported to be 67-86% during a race (128, 135, 136).

Motivation is an important factor in rowing training and performance. Mental preparedness, good coaching, positive thinking, and confidence in individual and in teammates' abilities are keys to successful rowing performance. Rowers' ability to resist feelings of mental and physical fatigue is enhanced by training.

Bottom line of literature review and conceptual framework of dissertation

Given iron's important role in aerobic metabolism, the high prevalence of iron depletion among active women, and its consequences relevant to endurance athletes (poor endurance performance), further study of female endurance athletes in-training

is warranted. There is little available evidence to show the effects of IDNA on training itself. There is even less evidence to show how the relationship between iron and performance is mediated by training.

As previously discussed, available evidence suggests that female athletes are more susceptible to depleted iron stores (resulting in IDNA) due to factors that can be attributed to the athletic environment (increased basal iron losses, inadequate dietary intake). The reviewed literature has also suggested that training itself may negatively affect iron stores. We know from animal and human studies that depleted iron stores results in IDNA, which affects oxidative capacity, endurance capacity, energetic or work efficiency, and local muscle fatigue. We also know from animal and human studies that training improves physical performance, as measured by energetic efficiency, $VO_2\text{max}$, endurance, and time to complete a time trial.

The conceptual framework used to conduct the studies in this dissertation is shown in Figure 2.4. The shaded portions of the framework will be the focus of this dissertation. Major indicators of iron status (sFer, sTfR, TBI), measures of physical performance ($VO_2\text{max}$, time to complete 4K time trial, and energetic efficiency), and a measure of training quality (training quality score) will be discussed. We hypothesized that IDNA and its consequences negatively affect training quality as measured by time spent training, intensity of sessions, and rest/recovery time, and that this negative effect of IDNA on training quality would inevitably negatively affect physical performance. We also hypothesized that training may negatively impact iron stores, which would negatively affect training quality, and result in poor physical performance.

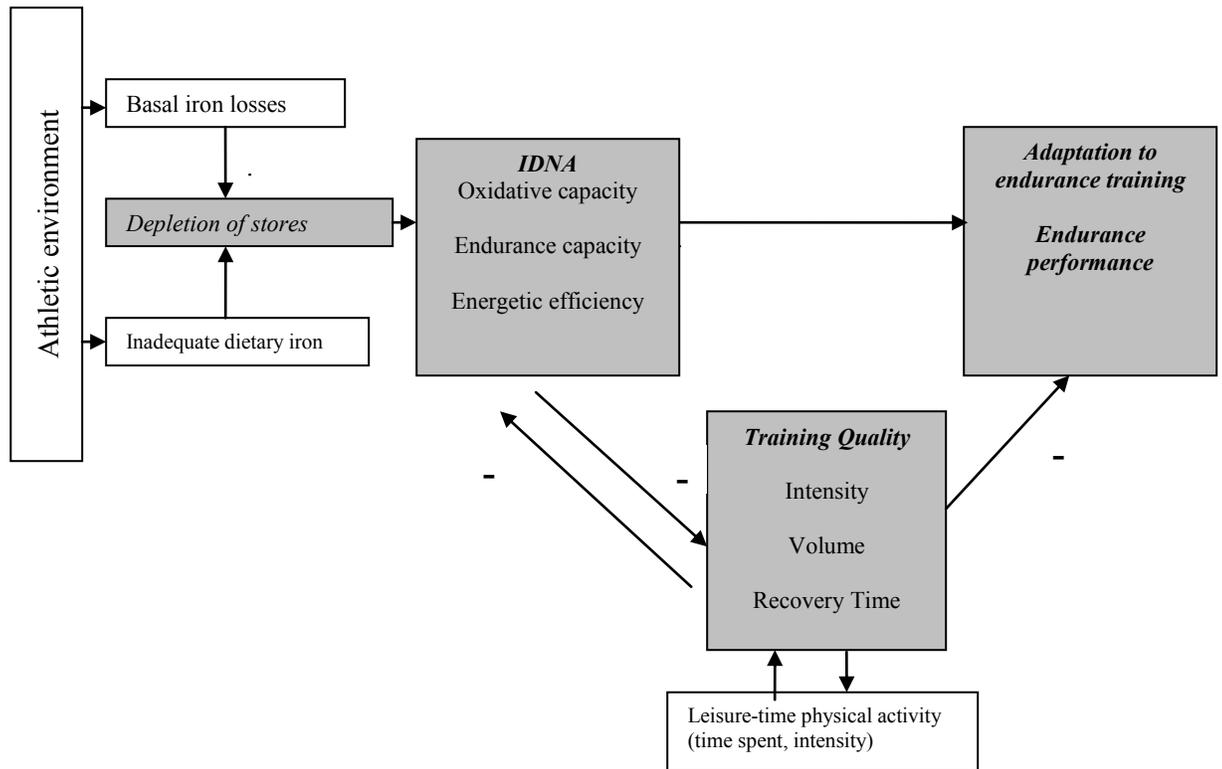


Figure 2.4. Conceptual framework to examine the relationships between iron status, training and performance

Using a randomized, placebo-controlled trial design, we used iron supplementation to remedy depleted iron stores in order to test the effects of IDNA on training and performance, as well as training on IDNA. We designed the study to see if iron supplementation (to replete iron stores) would prevent or reduce the negative effect of training on iron status, as well as allow for better training, and thus improved physical performance.

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CHAPTER 3

GENERAL METHODS

This study was designed to examine the relationship between iron status and adaptation to endurance training as related to rowing performance in a group of highly-trained female endurance athletes. The objectives of this study were meant to provide novel insight into the mechanisms mediating the relationship between IDNA and endurance training and performance. This chapter describes the design and analysis plan for the cross-sectional and RCT studies.

This study was conducted in three phases. During **Phase 1**, 165 female collegiate endurance athletes were screened to identify IDNA subjects (sFer <20 µg/l, Hgb ≥12 g/dL) for phases 2 and 3. **Phase 2** was a cross-sectional comparison of physical performance measures across a broad range of both skill levels (novice to Varsity) and iron status (normal and IDNA). A 6-week randomized, placebo-controlled iron supplementation trial was conducted during **Phase 3**. **During Phases 2 and 3**, we were interested in examining how IDNA rowers compensate for the effects of IDNA (e.g. local muscle fatigue, training intensity, reduced energetic efficiency, decreased endurance capacity). Both IDA and IDNA have been linked to decreased efficiency and work capacity in animals and humans, and it has been demonstrated that consequences of poor iron status include impaired adaptation to a training regimen (in previously untrained women) (1, 2).

We hypothesized that highly-trained female endurance athletes who are IDNA would not only have diminished physical performance as a consequence of IDNA, but that this reduced performance would result in part from impaired adaptation to their already-high training loads. We hypothesized that IDNA rowers would also train less hard as a consequence of, or in order to compensate for the effects of IDNA, compared to their non-IDNA (normal iron status) counterparts.

Prior to conducting this study, insufficient data existed on which to base nutritional supplement intake recommendations for iron in order to ameliorate IDNA in female collegiate endurance athletes. Currently, the National Collegiate Athletic Association (NCAA) does not include iron status screening as part of pre-season or follow-up medical screening (3). Data from this study provide novel information about iron status and training and performance for a specific group of female endurance athletes, and should inform recommendations for optimal iron nutrition status in this population.

Institutional Review Board (IRB) Approval

This study was approved by the Institutional Review Boards at the following colleges and universities: Cornell University (CU), Binghamton University (BU), Syracuse University (SU), Hobart and William-Smith Colleges (WS), and Ithaca College (IC, see Figure 3.1). The athletic directors and rowing coaches at all five schools were notified of the study prior to subject recruitment. Additionally, all coaches were interviewed prior to considering their rowing team eligible to participate in the study. Both phone calls to head coaches and in-person meetings with head and

Fall 2008 (WS, CU, IC), Spring 2009 (CU, IC, BU), and Fall 2009 (SU). All female rowers at CU and the surrounding colleges and universities mentioned (n=200) above were eligible to participate in the screening if over 18 years of age, non-smokers, and regularly-training with their rowing team. We recruited 165 female collegiate novice and varsity rowers between the ages of 18-30 years. Rowers were screened on the Cornell University campus, as well as on rowers' respective campuses (WS, IC, BU, SU). The number of subjects we chose to screen was based on the assumption that 30% would meet the criteria for IDNA and wish to continue participation in our supplementation trial.

Data collection: All data collection, including blood sampling and exercise testing of CU and IC rowers was conducted on the Cornell University campus in the Human Metabolic Research Unit (HMRU), a research facility operated by Cornell's Division of Nutritional Sciences (DNS). Blood was drawn in the HMRU by a trained phlebotomist (DMDV); laboratory samples were analyzed in the HMRU's Clinical Laboratory by a research lab technician (VS); exercise testing was conducted in the HMRU's Human Performance Laboratory by trained and experienced research personnel (DMDV); body composition assessments were completed in the HMRU's Body Composition Laboratory by trained research personnel (DMDV). Rowers outside of Ithaca, NY (WS, BU, SU) were screened and tested on their respective campuses (DMDV), times and locations determined by coaching staff (as agreed upon by each school's Human Subjects Committee/IRB).

Screening and testing off-campus worked out well, with the help of rowing coaching staff at SU, BU, IC and WS. Ithaca College rowers were screened on the IC

campus in the Wellness Center in order to minimize subject burden; all subsequent exercise and body composition testing was done in Cornell's HMRU. Weekly visits conducted by the researcher (DMDV) to IC students took place on the IC campus to maximize participation and compliance (capsule, log, and accelerometer drop-off and pick-up). Any IC student who did not have personal transportation to Cornell for testing was provided with or reimbursed for transportation. Rowers at WS, BU, and SU were both screened and tested on their respective campuses. To maximize participation, screening and testing in these locations took place in an area easily accessible to the rowers, and accommodating for our testing equipment (chair, table, metabolic cart, bioelectrical impedance analyzer, anthropometry, cleaning, etc). Testing took place in a gymnasium at WS and BU, and in an exercise physiology laboratory at SU (c/o T. Brutsaert's Human Performance and Body Composition labs, SU Exercise Science Department). Our metabolic testing equipment (metabolic cart, body composition equipment, etc) was brought to WS and BU to conduct all testing. Weekly visits were made to all rowers (usually before or after daily boathouse training session) to collect and distribute capsules, logs, and accelerometers (weekly visits to all schools: DMDV; occasional visits to CU rowers: KA, EC, KK).

A medical screening (NCAA-required) prior to our study pre-excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc). After NCAA medical clearance, and from a potential subject pool of ~250 female collegiate rowers, 165 subjects were recruited and iron status was screened during the first week of the conditioning phase of their competitive season (see Figure 3.2). A signed consent

form, explaining the details of the study and its different phases was obtained from each subject before any data collection (Appendix 2).

Each rower's iron status was assessed, and information on current dietary intake including iron intake, supplement use, menstrual status, usual physical activity, and eating habits and attitudes was obtained using questionnaires (described in subsequent chapters, and contained within Appendix 2-8). This screening of iron status occurred at the beginning of their training season (within the first week of training). Within one week of being screened, subjects were given the results of the iron status assessment (RBC, Hgb, Hct, sFer) as the benefit of participation (Appendix 10.8). Subjects participating in the laboratory testing were immediately given the results of all body composition and exercise testing variables as the benefit of participation in the cross-sectional and supplementation trials (Appendix 10.9). All anemics (Hgb<12.0 g/dL) were notified of their status immediately after blood test results (within 1 week of analysis) and were referred to campus health services for further instruction/monitoring.

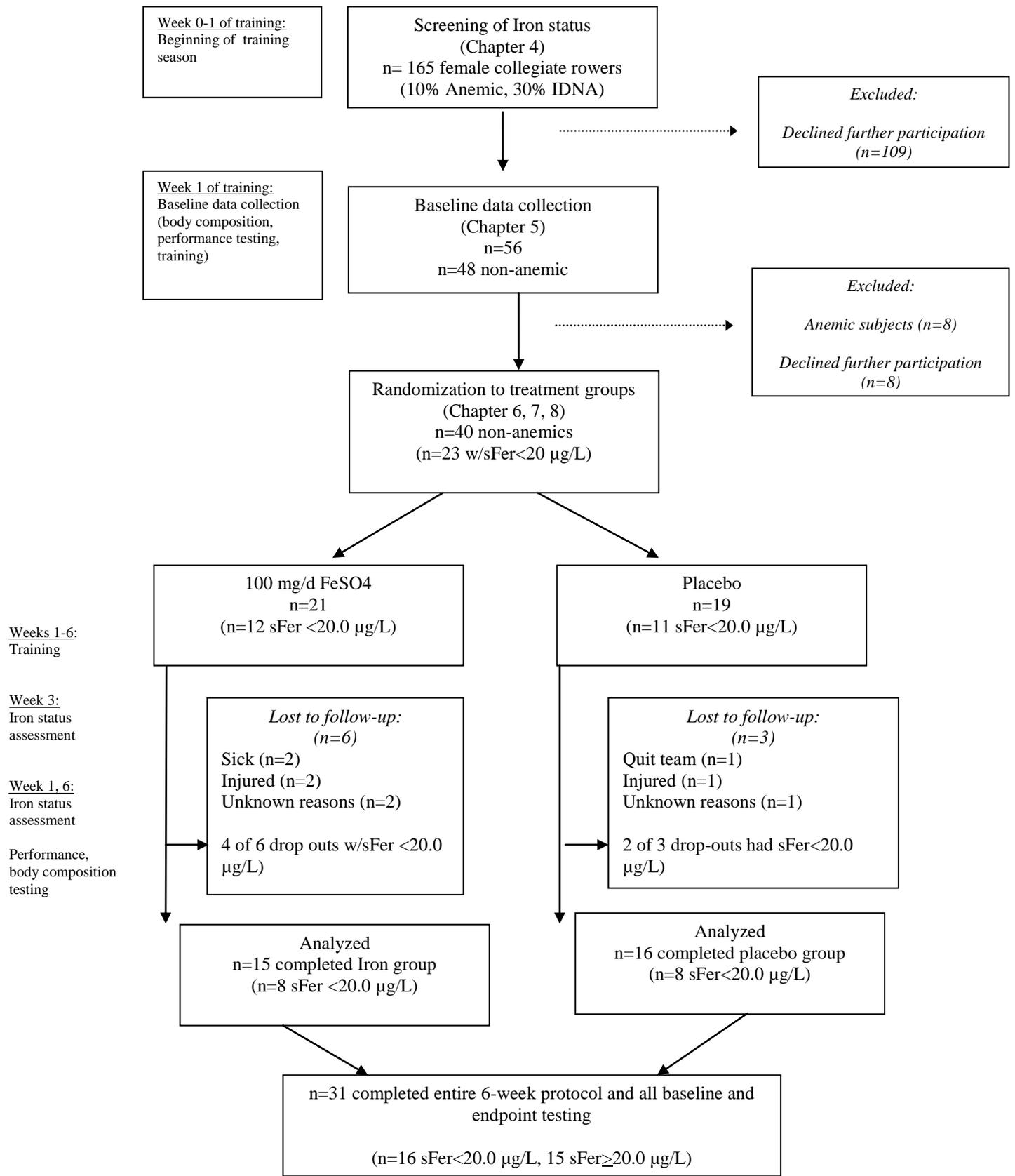


Figure 3.2. Time line and flow of subjects through 6-week supplementation study

Athletes would have been excluded from participating in the cross-sectional and supplementation trials if they had been clinically diagnosed with an eating disorder; were pregnant or lactating; had been taking iron supplements within 4 weeks of screening; or had any physical problems or presently take medications that would affect their ability to safely participate in exercise testing. Additional exclusions included: hemolytic anemia, exercise-induced asthma, excess alcohol consumption, drug use (casual and/or prescription medications), steroid use. No rowers who volunteered to participate in the laboratory testing were excluded based on these criteria.

Within one week of the screening, 48 non-anemic rowers volunteered to complete the baseline laboratory testing, after which forty rowers were randomly assigned to treatment groups. This study was a randomized, double-blind, placebo-controlled iron supplementation trial. Each subject was randomly assigned to a treatment group by a research assistant who was not involved in data collection or contact with subjects (DS, SK). Randomization was done by assigning each subject a random number, with even and odd numbers being assigned to either treatment group. After initial randomization, any imbalance in the distribution of treatment or representation of school or baseline iron status (sFer) was corrected by re-randomization.

Volunteers assigned to the placebo group were provided with capsules containing lactose filler; those assigned to the iron group were provided with capsules containing 50 mg ferrous sulfate (FeSO_4) for six weeks. Capsules used for both groups were identical. Both iron and placebo capsules were prepared by a Registered

Pharmacist (PharmD) at the Cornell University College of Veterinary Medicine Pharmacy (Ithaca, NY). The iron supplement capsules contained 50 mg FeSO₄ per capsule, and the placebo capsules contained lactose. The iron content of both placebo and iron capsules was analyzed via ICP mass spectrometry digestion by the USDA's Robert Holley Center for Agriculture and Health (Ithaca, NY). Twenty capsules were randomly selected for analysis from each of 2 batches. No differences in the average iron content were found between the two batches of capsules, however, the capsules contained 30% elemental iron (15.8 ± 0.5 mg elemental iron per capsule), which is greater than the 20% elemental iron expected from a typical FeSO₄ preparation.

Subjects were provided with 18 capsules each week, and were instructed to consume 2 capsules per day (total of 100 mg FeSO₄/day, ~30 mg elemental iron/day, based on the analysis of our capsules). Subjects were instructed to consume one capsule each with their morning and evening meals to minimize potential gastrointestinal side-effects, and with a glass of citrus juice to enhance iron absorption. This dose was selected based on previous findings both in and outside of our lab reporting the beneficial effects and minimal side effects of 100 mg FeSO₄ on iron status and performance in young, active women(4, 5).

Subjects' compliance with the iron treatment, as well as current health, menstrual status, and physical activity was assessed by daily logs (Appendix 10.10). Subjects were instructed to record the number of capsules they consumed daily in their log, even if they had consumed less than the prescribed daily amount. Additionally, weekly capsule counts were conducted by the researcher.

Thirty-one rowers completed the six-week trial, including endpoint laboratory assessments. No adverse events were reported during the trial. A total of nine subjects did not complete the trial due to injury, illness, separation from the rowing team, or personal/unknown reasons. Subjects were debriefed after completing the 6-week final assessments. They were informed of their treatment assignments and given laboratory, body composition, and physical fitness assessment reports, with recommendations for treating iron deficiency as necessary. All subjects who were ID and/or anemic at the end of the trial were given 30d of iron supplementation and/or referred to campus health services for further evaluation and/or counseling.

Analytical approach: Although the three phases of the study (screening, cross-sectional analysis of the baseline data, and the supplementation trial) share a common sampling frame, methodology, and are parts of a whole (RCT), the analyses were conducted separately and each tell a unique story. Measurements and assessment techniques used throughout all phases of this study will be described in the chapters that follow. This section outlines the analytical approach to each phase of the study.

Screening of iron status (Phase 1, Chapter 4): For this cross-sectional analysis, our main objective was to describe the iron status of our sample of female collegiate rowers at the beginning of a training season. Our main outcomes were indicators of iron status, as well as prevalence of anemia and iron deficiency with and without anemia. We also collected a self-reported measure of ergometer performance (reported 2K personal record (PR)) from the previous training/competitive season. For this performance outcome, the 149 non-anemic subjects were analyzed. We

hypothesized that iron depleted rowers would have slower 2K PRs compared to rowers with normal iron status.

Student's T-test and ANOVA were used to analyze differences between groups for iron status (sFer group, iron depleted versus normal, based on sFer cutoff of 20.0 $\mu\text{g/L}$) with respect to all variables measured. Pearson's correlations were used to examine associations between variables. To investigate the effects of iron status on our performance measure, multiple regression analysis (MIXED procedure with School as a random effect) between reported 2K time and sFer group status with potential confounders included as covariates in the regression models (height, years of experience). A significance level of $p < 0.05$ was used to test the main effects of the primary hypotheses, and $p < 0.10$ was the level of statistical significance used to test interaction effects.

Baseline laboratory assessments (Phase 2, Chapter 5): For this cross-sectional analysis, our main outcomes were measures of physical performance that were assessed in the laboratory at the beginning of the training season. These included 4K time trial (TT) time, peak oxygen consumption reached during the 4K TT (VO_2 peak), and gross energetic efficiency (EF). This analysis included the 48 non-anemic rowers that completed all laboratory testing, as well as a 7-day training log at baseline. We hypothesized that rowers with depleted iron status would have lower VO_2 peak, be less energetically-efficient, and have a slower 4K TT time compared to rowers with normal iron status. We also hypothesized that iron-depleted rowers would train less hard during that first week of training, meaning that they would spend less time training at a lower intensity compared to rowers with normal iron status.

Student's T-test and ANOVA was used to analyze differences between groups of non-anemics (*Normal*: sFer \geq 20.0 μ g/L vs *Depleted*: sFer $<$ 20.0 μ g/L) with respect to all variables measured. Pearson's correlations were used to examine associations between variables. To investigate the plausible effects of iron status on endurance performance, multiple regression analyses (MIXED procedure with School as a random effect) of the relationship between measures of physical performance assessed in the laboratory (VO₂ peak, gross EF, 4K TT time) and total body iron stores (sFer, sTfR, TBI) was conducted, controlling for potential confounders. Covariates used in the development of regression models included training group (based on session RPE cutoff of 3200, low vs high); height, fat-free mass, deviation from prescribed work rate, and average work rate during the 4K TT. A statistical significance level of $p < 0.05$ was accepted for testing main effects. We also explored the cross-sectional relationship between iron status and training on performance outcomes at the beginning of the training season using sFer group-by-training group. A $p < 0.10$ was considered significant for testing these interaction effects.

Randomized, placebo-controlled iron supplementation trial (RCT, Phase 3, Chapters 6, 7, 8): At the end of the RCT, change from baseline in iron status, training quality, and measures of physical performance mentioned above were our main outcomes. Supplementation trial analyses were conducted on an "as-treated" basis, including all randomized subjects, regardless of adherence or dose consumed. A $p < 0.05$ was considered significant for testing main effects, and $p < 0.20$ for exploring interaction effects. Iron status data was log-transformed where appropriate prior to analyses.

Independent Student's *t*-test was used to test group differences at baseline, since randomization was not a guarantee that both treatment groups were identical in all respects. The confounding potential of any factor that was significantly different between groups was further tested using correlation analysis. All potential confounders were included as covariate in subsequent analyses.

Repeated measures ANOVA were used to test group and time effects as well as group-by-time interactions for measures of iron status and performance. Regression analysis with baseline measurements as covariates were used to analyze group differences in change in iron status or physical performance (endpoint minus baseline), as well as other potential confounding or mediating factors. All models were tested for β not equal to zero at $p < 0.05$ on a one-tailed test.

The effects of change in iron status on change in other outcome variables were analyzed by multiple linear regression analysis (GLM, MIXED procedure with School as random effect), controlling for baseline outcome values and iron status as well as other potential confounding or mediating factors.

Change from baseline (outcome variables)=
 β_0 (constant) + β_1 Baseline + β_2 group (S vs P) + $\beta_C X_C$ (potential confounders)

To test for plausibility, a dose-response in sFer was tested for the iron supplemented group by examining the correlation between change in sFer and dose of iron capsules consumed, showing that the changes in outcome variables were due to the iron treatment (those in iron group who took more capsules should have exhibited greater change in iron status). Analyses were also conducted to test potential to

benefit, or baseline iron status by change in iron status (those with lowest baseline iron status should have exhibited greater response to treatment). Similar plausibility testing was conducted for performance and training outcomes (dose-response, baseline values).

Change from baseline (outcome variables) =
 β_0 (constant) + β_1 Baseline outcome (pre-Tx) + β_2 change-Iron status (post-Tx iron status minus baseline iron status) + $\beta_C X_C$ (confounders)

The relationship between iron status and performance was hypothesized to be mediated by training. Change in performance outcome variables with change in iron status, as well as change in training status were conducted.

Change from baseline (outcome variables)=
 β_0 (constant) + β_1 Baseline outcome (pre-Tx) + β_2 change-Iron status (post-Tx iron status minus baseline iron status) + β_3 change in mediator (final value minus baseline value) + $\beta_C X_C$

Sample size estimation: Using a significance level $p < 0.05$, 80% power, the following is a summary of effect sizes (Table 3.1) that we had expected with iron supplementation (Treatment vs Placebo) based on previous research with non-athletic women with good compliance ($n=20/\text{group}$) in our lab (2, 6). Based on previous reports of 30% IDNA in active females, we needed to screen approximately 150 rowers to reach a sample size of 20-25 rowers per treatment group.

Table 3.1 Effect sizes of important outcome variables used to calculate sample size

Main Outcome Measures	Expected effect size (SD units)	Control group SD	Sample size needed per group
Post-tx sFer ($\mu\text{g/L}$)	1.12	4.0	11
Change in TT time (min)	0.70	2.2	26
Change VO_2max (ml/kg/min)	0.65	8.7	29

Effect size and sample size calculator:

<http://www.danielsoper.com/statcalc/default.aspx>

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CHAPTER 4

IMPACT OF IRON DEPLETION WITHOUT ANEMIA ON TRAINED ENDURANCE ATHLETES AT THE BEGINNING OF A TRAINING SEASON: A STUDY OF FEMALE COLLEGIATE ROWERS IN CENTRAL NY STATE

Abstract

The objective of this study was to determine the impact of iron depletion without anemia in a sample of female collegiate rowers at the beginning of a training season (August 2008, January 2009, & September 2009). One-hundred and sixty-five female collegiate rowers from five colleges and universities in central NY state participated in a screening of iron status. Blood hemoglobin (Hgb), serum ferritin (sFer), and soluble transferrin receptor (sTfR) were measured to determine prevalence of iron depletion and anemia. Rowers' habitual moderate and vigorous physical activity, as well as their best time (PR) to complete a 2K simulated race during the previous three months were self-reported. Sixteen rowers (10%) were identified as anemic (Hgb<12.0 g/dL), nine of whom (5% of total) were iron-depleted (sFer<20.0 µg/L). Thirty percent (N=44) of the non-anemic rowers were identified as iron depleted without anemia. Multiple regression analyses revealed that sFer group status (based on sFer cutoff of 20.0 µg/L) was a significant ($p<0.001$) predictor of rowers' reported 2K performance times (mean 7 min 49 seconds) after controlling for height and years of rowing experience. Iron depleted rowers reported 2K times ~21 seconds slower ($p<0.004$) compared to rowers with normal iron status. Iron depletion without anemia is a prevalent problem among female endurance athletes. A sFer cut-off of 20.0 µg/L is clinically useful to identify and treat non-anemic iron depletion at the

beginning of a training season, and improving sFer status may improve rowing performance.

Introduction

Iron deficiency (ID) is the most prevalent nutrient deficiency in the world. In the United States, iron deficiency with anemia (IDA) affects 3-5%, and iron deficiency without anemia (IDNA) affects ~16% of premenopausal women (1). Changes in energy metabolism and physical work capacity have been described in humans and animals with iron depletion (2-4). Compared to their sedentary counterparts, female athletes are more susceptible to iron depletion without anemia (IDNA, 25-35%), which is 5-7 times more prevalent than IDA (5-10). Though the exact mechanism is unknown, the increased prevalence of IDNA in active or training females may be due to one or a combination of the following factors: hemolysis (foot strike, impact); increased blood loss (gastrointestinal tract, hematuria, sweat); poor dietary iron intake; or altered intestinal iron absorption, including the effects of inflammation due to training (11-13).

Collegiate rowers train for and compete in racing competitions that last <30 min (short-duration and high-intensity exertion), during which oxidative metabolism is the main energy pathway. It has been shown that the activity of iron-dependent enzymes and cytochromes needed for oxidative metabolism are decreased in animals (14) and humans (15) with IDNA, which leads to impaired endurance performance. Thus, consequences of IDNA particularly relevant to endurance athletes include reduced endurance capacity and energetic efficiency (16), and increased local muscle fatigue (17).

Serum ferritin (sFer) is the most common index of body iron stores, and reflects iron stored in the liver. sFer can be elevated in an inflammatory state (e.g. infection, post-exercise), however, an inflammatory marker such as C-reactive protein (CRP) or α -1-acid glycoprotein (AGP) can help identify individuals with false-positive iron depletion due to inflated sFer (18-20). Soluble transferrin receptor (sTfR), a trans-membrane protein regulated by cellular iron status, reflects ID at the tissue level and is a more sensitive index of functional ID. Unlike sFer, sTfR is unaffected by inflammation, and has been shown to have lower within-subject variability in intensely training athletes (5). Total body iron (TBI, mg/kg) can be calculated using both sFer and sTfR (21), though in a population with a low prevalence of anemia, calculated TBI is driven by sFer.

The purpose of this study was to examine the prevalence of IDNA among female collegiate rowers at the beginning of a training season, and determine the association between iron status and reported rowing performance.

Methods

Recruitment of subjects: This study was approved by the Institutional Review Boards of the following colleges/universities: Cornell University, Binghamton University, Syracuse University, Hobart & William Smith Colleges, and Ithaca College. Rowers were recruited over a period of three years (Spring 2006, Fall 2008, Spring 2009, and Fall 2009). Varsity and second-semester novice female rowers who were >18 years of age and able to begin regular training for their sport were eligible to participate in the screening. Subjects were recruited at the beginning of the conditioning phases of their competitive rowing seasons (upon arrival to campus post-

summer and post-winter break). Training activities during this time included general aerobic conditioning (cycling, running, rowing on ergometer), resistance training, and high-intensity rowing training.

Team coaches were instrumental in recruitment and screening efforts. A medical screening (NCAA-required) prior to our screening excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc). All rowers provided written voluntary informed consent prior to participating in the study. A total of 165 female rowers out of 199 eligible rowers completed the iron status screening. Subjects received iron status results as a benefit of participation, along with referral/recommendations to improve iron status as necessary.

Measurements: After NCAA medical clearance, iron status variables were measured for each subject and demographic, as well as information on current dietary supplement use, health and menstrual status, and habitual physical activity. Height and weight were self-reported, and were later validated with measured height and weight in a sub-sample of 48 subjects who participated in an intervention trial (height: $r=+0.98$, $p<0.001$; weight: $r=+0.99$, $p<0.001$, and no systematic bias). Time spent in light/moderate and vigorous physical activity was quantified via self-report (habitual endurance training, and leisure-time physical activity (LTPA) frequency, intensity, type, and duration). Performance was assessed via self-reported best times (PRs) to complete a 2K simulated race on the ergometer from the previous season (2-3 months prior to iron status screening). Reported 2K times were also later validated with

measured performance in the aforementioned subsample of 48 rowers (VO_{2peak} , L/min: $r=-0.69$, $p<0.001$; 4K time, min: $r=+0.64$, $p<0.001$).

Iron status variables measured immediately after non-fasting venous blood sampling (antecubital venipuncture, into two evacuated tubes with EDTA and serum-separator) included hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC, Beckman Coulter, Fulerton, CA); sFer (Immulite 2000); soluble transferrin receptor (sTfR, ELISA, Ramco Laboratories, Stafford, TX); and α -1-acid glycoprotein (AGP, radial immunodiffusion plate, Kent Labs, OR). Rowers were classified as either iron depleted ($sFer<20 \mu\text{g/L}$) or normal ($sFer\geq 20 \mu\text{g/L}$). All anemic subjects ($N=16$) were notified of their status immediately after blood test results (within 1 week of analysis) and referred to their respective campus health services for further instruction/monitoring or treatment.

Data Analysis: All data analysis on 149 non-anemic subjects was completed using SPSS (version 18.0, SPSS Inc, Chicago, IL). Descriptive statistics are presented as means \pm standard deviations (SD). Student's T-test and ANOVA were used to analyze differences between groups for iron status (iron depleted versus normal) with respect to all variables measured. When a significant overall difference was detected, Bonferroni's post-hoc analysis was used. Pearson's correlations were used to examine associations between variables. To investigate the effects of iron status on physical performance, multiple regression analysis between physical performance (2K time) and sFer group status (based on sFer cutoff of $20.0 \mu\text{g/L}$) with potential confounders included as covariates in the regression models (variables correlated with physical performance, or significantly different between iron status groups) were included as

covariates. A significance level of $p < 0.05$ was used to test the main effects of the primary hypotheses, and $p < 0.10$ was the level of statistical significance used to test the interaction effects.

Results

Sixteen rowers (10%) were identified as anemic ($Hgb < 12.0$ g/dL) and eliminated from subsequent analyses of IDNA subjects. Results of the iron status screening of non-anemic rowers are presented in Table 4.1. Thirty percent ($N=44$) of the non-anemic rowers were found to be iron depleted ($sFer < 20.0$ μ g/L), and 12% ($N=18$) were clinically iron deficient ($sFer < 12.0$ μ g/L). There were no significant differences between rowers with depleted or deficient iron status and those with normal iron status in any demographic variable. Nineteen non-anemic rowers (13%) had high values of sTfR (> 8.5 mg/L) and 10% ($N=15$) had TBI < 0 mg/kg, indicating severe iron deficiency. Nineteen percent of screened subjects had a previous history of anemia or physician- diagnosed iron deficiency.

Table 4.1. Iron status (mean±SD) of non-anemic female collegiate rowers (between schools) at the beginning of training season

Iron status indices	School A (CU) N=60	School B (IC) N=39	School C (BU) N=18	School D (WS) N=16	School E (SU) N=16	Total (All) N=149
Serum ferritin (sFer, µg/L)	31.9±22.0	26.4±14.1	31.3±23.4	42.0±21.1	51.2±28.0*	33.6±22.1
Log sFer (µg/L)	1.4±0.3	1.4±0.3	1.4±0.3	1.6±0.2	1.6±0.3	1.4±0.3
Hemoglobin (Hgb, g/dL)	13.1±0.7	13.3±0.7	13.2±0.6	13.1±0.7	13.6±0.7	13.2±0.7
Red Cell Distribution Width (RDW, %)	13.1±1.0	13.3±1.2	13.0±2.1	12.5±0.7	12.6±0.4	13.0±1.2
Hematocrit (Hct, %)	40.2±2.3	41.3±2.3	39.8±1.5	41.3±1.9	41.7±2.2	40.7±2.2
Mean Cell Volume (MCV, fL)	88.3±3.2	89.1±3.4	88.5±4.4	89.2±4.2	90.5±1.7	88.8±3.5
Soluble transferrin receptor (sTfR, mg/L)	6.4±1.6	6.9±2.1	6.1±2.0	5.8±1.4	7.1±2.7	6.5±1.9
Total Body Iron (TBI, mg/kg)	3.5±3.3	3.1±2.7	3.6±3.4	5.4±1.5	5.3±2.4	3.8±3.0
Number and prevalence (%) of iron depletion and normal iron status between schools						
Normal (sFer>20.0 µg/L)	40 (67)	25 (64)	12 (67)	15 (94)	13 (81)	105 (70)
Iron depleted (sFer<20.0 µg/L)	20 (33)	14 (36)	6 (33)	1(6)	3 (19)	44 (30)
Iron deficient (sFer<12.0 µg/L)	9 (15)	5 (13)	4 (22)	0 (0)	0 (0)	18 (12)
sTfR >8.5 mg/L	4 (7)	9 (23)	3 (2)	1 (6)	2 (13)	19 (13)
TBI <0.0 mg/kg	7 (12)	5 (13)	2 (11)	0 (0)	1 (6)	15 (10)
AGP>140.0 mg/dL	1 (2)	2 (5)	0 (0)	0 (0)	0 (0)	3 (2)

*Significantly different from Schools A and B (p=0.014 and 0.04, respectively).

On average, rowers were 19.7 ± 1.2 years of age, 170.7 ± 7.3 cm tall, and weighed 69.5 ± 8.1 kg (BMI 23.7 ± 3.3 kg/m²). Across the sample, rowers had 3.0 ± 2.3 years rowing experience; a previous season 2K time of 7 minutes and 48 seconds (469 ± 25 sec); and spent 6.4 ± 2.2 hours per week in combined moderate and vigorous physical activity. On average, School E rowers reported spending more time participating in vigorous physical activities (5.6 ± 3.3 hr/week) compared to rowers at Schools A and C (2.7 ± 2.3 and 2.7 ± 2.0 hr/week, respectively). Compared to the rest of the sample, rowers at School C reported slower 2K times (8 min and 12 sec, or 493.4 ± 25.2 sec). Rowers at schools A and E were taller (175 cm), faster (7 min and 42 sec, or 460 sec 2K time), and more experienced (3.8 y) than the other schools in the sample.

Simple correlations revealed that reported time spent in light-to-moderate physical activity per week was positively associated with BMI ($r=0.23$, $p=0.020$) and years of rowing experience ($r=0.29$, $p=0.03$). As expected, height and weight were negatively correlated with 2K performance time ($r=-0.51$ and -0.39 , respectively, $p<0.001$). Rowers with less experience were more likely to have lower sFer ($r=0.30$, $p=0.01$). Total body iron was not correlated with 2K PR ($r=-0.06$, $p=0.55$) or weekly time spent in vigorous ($r=0.05$, $p=0.65$) or light-to-moderate ($r=0.12$, $p=0.28$) physical activity. There was no significant correlation between supplement use and TBI ($r=-0.26$, $p=0.77$). Rowers reportedly taking a vitamin/mineral supplement (33% of sample) were more likely to have a higher BMI ($r=0.18$, $p=0.03$) compared to those who did not report taking a dietary supplement.

Multiple regression analyses revealed that sFer group, height, and years of rowing experience were each a significant predictor of 2K performance time ($p < 0.05$). For these non-anemic subjects, Hgb was not a significant predictor of 2K time and was not included in the multiple regression models, and there were no significant interactions between iron status and the amount of time spent in physical activity. On average, 2K times were 21 seconds slower for those rowers with iron depletion ($sFer < 20.0 \mu\text{g/L}$) compared to rowers with normal iron status ($p = 0.004$, see Figure 4.1).

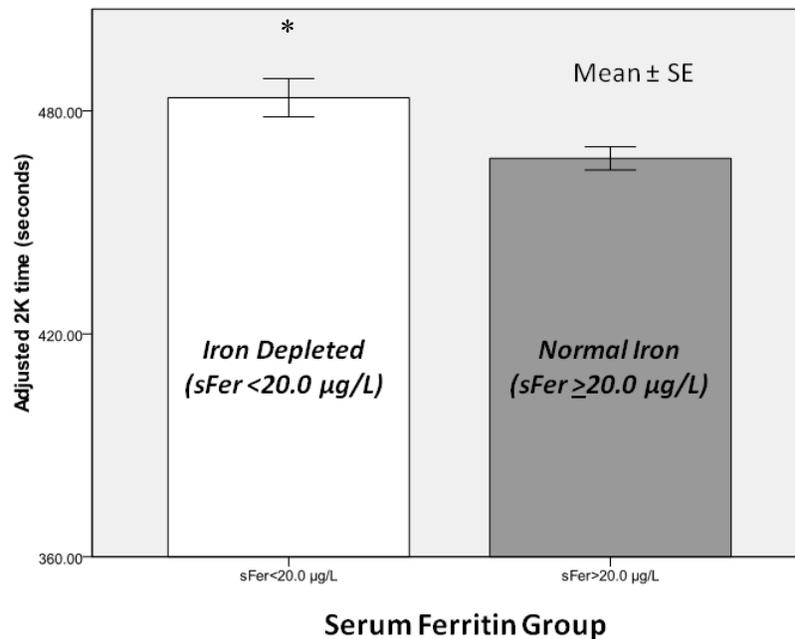


Figure 4.1. The impact of IDNA on rowers' reported 2K performance (means adjusted for years of rowing experience and height, $p = 0.004$). *Depleted rowers ($sFer < 20.0 \mu\text{g/L}$) significantly different from Normal rowers ($sFer \geq 20.0 \mu\text{g/L}$), $p = 0.004$.

Discussion

Our sample of collegiate female rowers is one of high risk for iron depletion due to their age and high training load, among other factors. Although the exact mechanism by which endurance training results in decreased iron status remains unknown, possible explanations include poor dietary intake (7, 22-24); increased iron loss in sweat and urine (25); increased hemolysis (26, 27); the acute phase response (27, 28); or plasma volume expansion (29). With the exception of the acute phase protein AGP (which was not used to exclude subjects from the analysis), these factors were not measured in the current sample.

Strategies to easily screen and improve iron status may be useful for female endurance athletes at the beginning of a training season because data suggest that iron status of very active women may decrease with increased levels of physical training over time (30-33). In a recent study, McClung et al (30) examined 94 female US Army recruits before and after 9 weeks of Basic Combat Training (BCT) and found that sFer decreased by 20% ($p < 0.01$). In other longitudinal (5 weeks to 6 y) studies of female athletes, sFer decreased by ~25% after training (32, 33).

Early screening and iron supplementation, both of which have been shown to improve resistance to fatigue and endurance capacity in IDNA non-athletes (16, 17), may be beneficial to female endurance athletes at the beginning of a season to thwart further decrements in iron status throughout the training and competitive seasons. Given the modest relationship between early season iron status and recent performance observed in this study, improving iron status may improve endurance athletes' adaptation to training, as well as their performance in competition.

The cross-sectional design of this study limits our ability to examine factors that account for differences in iron status between schools, which could be the result of differences in iron-related variables other than rowing training. Not all potential confounders could be measured in this survey. Due to logistic considerations of the current survey, a self-reported measure of performance (2K PR) during the previous 2-3 months was used as an outcome, and although the self-reported measure was highly-correlated with lab-derived values in a subsample of 48 rowers, results should be interpreted with caution. Although a limitation, the observed effect of IDNA on a non-concurrent measure of physical performance is consistent with laboratory data showing that IDNA impairs endurance performance in non-athletic women (16, 34). It would be important to confirm these results in a longitudinal study, preferably following the same rowers periodically throughout both seasons of the academic year. Future research should examine the prevalence, risk factors, and mechanisms of IDNA in female athletes, including plasma markers of hemolysis, accurate measures of athletes' menstrual blood loss, dietary iron intake/absorption, as well as other markers of iron metabolism, such as hepcidin.

Conclusions: The 30% prevalence of IDNA among the female rowers in this study was similar to that previously reported for other female athletes participating in endurance sports, as well as female soldiers (5-10). Results from this study add to the evidence that iron status is an important issue facing female endurance athletes at the beginning of a training season. The prevalence of iron depletion is higher in female endurance athletes compared to the general population of young women due to many factors. Female endurance athletes should be screened for iron deficiency and

depletion using serum ferritin cut-off of 20.0 $\mu\text{g/L}$ to identify iron depletion before it leads to anemia or frank iron deficiency, thus reducing the adverse affects that iron depletion may have on their training and performance. Athletes with a history of anemia or iron deficiency should receive counseling regarding supplementation and food choices, as well as serial monitoring of their iron status (Hgb, sFer).

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CHAPTER 5

HOW IS IRON STATUS ASSOCIATED WITH ENDURANCE PERFORMANCE AND TRAINING IN FEMALE COLLEGIATE ROWERS?

Abstract

Introduction: Studies in both animals and humans have shown a relationship between iron depletion without anemia (IDNA) and physical performance. Consequences of IDNA relevant to endurance athletes include reduced work capacity, endurance, and energetic efficiency. We conducted a cross-sectional study to investigate the relationship between iron status and endurance performance and training in non-anemic female collegiate rowers.

Methods: The iron status (hemoglobin, serum ferritin, soluble transferrin receptor) of 165 female rowers from five colleges and universities in central New York, USA was assessed at the beginning of a training season. Twenty-seven percent (n=44) of rowers were identified as IDNA (sFer<20.0 µg/L) and 10% as anemic (n=16, Hgb<12.0 g/dL). Forty-eight (n=24 *Normal*, n=24 *Deplete*) non-anemic rowers volunteered to have their body composition measured and physical performance assessed (VO₂ peak, 4K time, gross energetic efficiency). Rowers also recorded their training (time, type and intensity) for 7 days, and daily training load was calculated using the session RPE method (training duration-by- intensity rating).

Results: There were no significant differences between the two iron status groups in any of the potential confounders of the association between iron status and performance that were tested. Compared to rowers with *Normal* iron status, *Depleted* rowers trained ~10 minutes less per day (p=0.02), and had a 0.3 L/min lower VO₂peak

($p=0.03$). With multiple regression analysis both sFer and training group (high versus low based on session-RPE cut-off of 3200) were significantly positively related to VO_2 peak. Less highly-trained rowers with poor iron status had a lower VO_2 peak (-0.32 L/min, $p=0.02$), and were less energetically-efficient (-1.7%, $p=0.09$) compared to more highly-trained rowers with poor iron status, and improved performance with improved iron status was only observed in less well-trained subjects.

Conclusion: Impaired iron status is a prevalent problem among female endurance athletes. The performance of less highly-trained rowers was poorer among those with low iron status than that of more highly-trained rowers. IDNA may prevent rowers from training as hard, directly impacting their performance.

Key words: *iron depletion, athletes, physical performance, VO_2 peak, energetic efficiency*

Iron status and endurance performance in female collegiate rowers

Introduction

Iron deficiency (ID) is the most prevalent nutrient deficiency in the world, including the US, where iron deficiency with anemia (IDA) affects 3-5%, and iron deficiency without anemia (IDNA) affects ~16% of young women (1). Changes in energy metabolism and physical work capacity have been described in humans and animals with iron depletion (2). Compared to their sedentary counterparts, female athletes are more susceptible to iron depletion, with prevalence as high as 25-35% (3-7). Although the exact mechanism is unknown, the increased prevalence of IDNA in active and training females may be due to a combination of factors, including: hemolysis (foot strike, impact); increased blood loss (gastrointestinal tract, hematuria, sweat); change in iron absorption due to inflammation of training; and/or poor dietary iron intake (8-11).

Iron plays an essential role in oxygen transport and energy production during aerobic exercise. Individuals with anemia (hemoglobin (Hgb) <12.0 g/dL) experience decreased maximal oxygen consumption (VO_{2max}), an indicator of physical work capacity (2). Individuals with IDNA (serum ferritin (sFer) <20.0 μ g/L, Hgb >12.0 g/dL) are expected to have adequate O_2 -carrying Hgb but impaired O_2 utilization, and thus suffer the functional consequences of tissue iron depletion, which results in reduced physical performance (12).

Despite the inability to identify the exact mechanism in humans, endurance capacity, energetic efficiency, and time trial (TT) performance have been shown to be impaired in laboratory studies of IDNA human research subjects (13-16). Studies of

how iron status affects non-anemic endurance athletes are limited, but research suggests that female endurance athletes and military soldiers with IDNA also have impaired physical performance (17-21). It has been suggested in animal and human studies that iron status is related to physical training, but the causal direction of this relationship is unclear (22-28). In a sample of 149 non-anemic female rowers, we found that sFer was a significant predictor of reported 2K Personal Record (PR) time, and that iron-depleted rowers reported 2K times that were ~21 sec slower ($p < 0.001$) compared to rowers with normal iron status (*Chapter 4*). The strong relationship between rowers' iron status and reported performance, as well as the limitation of self-reported performance led us to perform the current study in which we were able to examine the relationship between iron status and performance measures assessed in a laboratory setting.

The objective of this study was to examine the effects of iron depletion without anemia (IDNA, sFer < 20.0 $\mu\text{g/L}$, Hgb ≥ 12.0 g/dL) on female collegiate rowers' endurance training and exercise capacity as assessed by VO_2peak , gross energetic efficiency, and time to complete a 4K time trial (TT), as well as reported training intensity and duration. We hypothesized that rowers with IDNA would have a lower VO_2peak , be less energetically efficient, have poorer 4K TT performance, and train less than rowers with normal iron status.

Methods

Recruitment of subjects: This study was approved by the Institutional Review Boards of the following colleges/universities: Binghamton University, Cornell University, Hobart and William-Smith Colleges, Ithaca College, and Syracuse

University. All rowers provided written informed voluntary consent prior to participating in the study. Subjects were recruited at the beginning of the conditioning phases of their competitive rowing seasons (fall 2008, spring 2009, and fall 2009, upon arrival to campus post- summer and post-winter break). All varsity and second-semester novice female rowers were eligible to participate in the screening if greater than 18 years of age, non-smoking, and were able to begin regular training for their sport. Training activities during this time included general aerobic conditioning (cycling, running, rowing on ergometer), resistance training, and high intensity rowing.

Team coaches were instrumental in recruitment and screening efforts. Initial meetings with each coach were conducted prior to the start of each season to discuss the study and assess coach/team interest. Coaches approached his/her team during their initial pre-season team meetings to assess interest in participating in the iron status screening. A medical screening (required by the National Collegiate Athletic Association, NCAA) prior to our study excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc).

A total of 165 female rowers from the five schools completed the iron status screening. All 149 non-anemic subjects with and without iron depletion were invited to participate in the physical performance and body composition testing. A total of 24 IDNA rowers and 24 rowers with normal iron status participated in the present study (n= 12 Binghamton, 14 Cornell, 9 Ithaca, 8 Syracuse, and 5 William-Smith). Subjects received iron status, body composition, and fitness testing results as a benefit of

participation, along with referral/recommendations to improve iron status as necessary.

Assessment of iron status: Iron status variables measured from non-fasting venous blood samples (antecubital venipuncture, into two evacuated tubes, EDTA and serum-separator) included hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC, Beckman Coulter, Fulerton, CA); serum ferritin (sFer, Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL); soluble transferrin receptor (sTfR, Ramco Laboratories, Stafford, TX); alpha-1-acid glycoprotein (AGP, radial immunodiffusion plate, Kent Labs, Bellingham, WA). Total body iron (TBI, mg/kg) was calculated using the ratio of sTfR to sFer as described by Cook et al (29). Iron status was analyzed immediately after blood sampling. Rowers were classified as either iron depleted (sFer<20.0 µg/L), normal (sFer≥20.0 µg/L), or anemic (Hgb<12.0 g/dL). All anemics were notified of their status immediately after blood test results (within 1 week of analysis), referred to their respective campus health services for further instruction/monitoring, and excluded from further participation in the study. All laboratory assays were done in the Human Metabolic Research Unit at Cornell University.

Questionnaires: Information about current dietary supplement use, health and menstrual status, usual physical activity, and eating habits and attitudes was obtained using questionnaires and a 7-day food diary (see Appendix 3-6). Rowing training regimen, as well as leisure-time physical activity (LTPA, Appendix 8) outside of rowing training was quantified for 7 days via detailed training and activity records (Appendix 11). Questions in the daily log addressed sleep and nap duration and

quality, soreness and fatigue, and training/physical activity frequency, intensity, time, and type. Questions were presented in the format of a Visual Analog Scale (VAS) (30). Subjects were asked to rate each question by placing a solid vertical line on a 100mm scale anchored by opposing descriptors (see Figure 5.1). All VAS questions were “scored” by measuring the rating with a ruler (mm).

Daily Log Instructions

This training log should be completed on a ***daily basis*** for the next 7 days. Initial use of this log may take up to 5 minutes/day. For some questions, please rate each factor, as you feel ***today*** by placing a ***solid vertical line*** on the scale.

Example: Happy: How happy do you feel right now?

Not at all happy _____ | _____ Extremely happy

Figure 5.1. Visual Analog Scale (VAS) instructions and format of daily training log

The session rating of perceived exertion (RPE) method (31) was used to quantify daily training load. VAS Intensity score for each training session was multiplied by training session duration (time). This method has been used by others to quantify training in athletes (32-34). We validated the session RPE method using our VAS-format with the summated heart rate zone method (35) during two weeks of training on a separate sample of thirteen female rowers and found a significant positive correlation between the two methods ($r=0.85$, $p<0.001$, Appendix 1). In the current study, rowers were classified as either “High” trainers or “Low” trainers based on the session-RPE cutoff of 3200, which was the median (50th percentile) Session RPE of the sample.

Body composition: Anthropometric and body composition measurements were determined at the site of exercise testing. Body weight and height were measured with standard procedures and equipment (36). For athletes for whom it was accessible (n=31), body fat and composition was assessed via air-displacement plethysmography (BodPod, Life Measurement, Inc, Concord, CA). For all subjects, percent body fat was calculated from tricep, suprailiac, and thigh skinfold thickness (SF, Lange, Cambridge, MD) (37) and bioelectrical impedance analysis (BIA, RJL Systems, BIA-101) (38). The Siri equation (39) was used to calculate percent fat from body density. For those athletes without access to the BodPod (n=17), an average of their percent body fat values calculated from BIA and SF was used. In the sample with both BodPod and BIA-SF average (n=31), the two methods were highly correlated ($r=0.83$, $p<0.001$), and not significantly different from each other (paired t-test, $p=0.40$). The mean difference between percent body fat calculated from BodPod and the BIA-SF average was $-0.48\pm 3.15\%$ (95% confidence interval of difference: $-1.64, +0.67$). There were no significant differences in either body fat measurement method between schools.

Physical performance testing methods: Physical fitness and endurance performance was assessed using a rowing ergometer (Concept2, Morrisville, VT) equipped with a digital readout monitor (PM2), displaying work (watts, W), stroke rate (spm), distance (m), and elapsed time (min:sec). A computerized metabolic cart (TrueMax 2400, ParvoMedics, Salt Lake City, Utah) was used to measure VO_2 and other physiological measures during all testing. Concentrations of O_2 and CO_2 in

expired air were analyzed with gas analyzers (which are routinely calibrated with gases of known O₂ and CO₂ concentration). Respiratory volume (V_E) was measured with a respiratory pneumotachograph (Fitness Instrument Technologies, Farmingdale, NY) through a two-way breathing valve (Hans Rudolph, Kansas City, MO).

Energy expenditure was assessed via indirect calorimetry during exercise testing using a standard protocol that measured expired gases for V_E, VO₂, VCO₂, and respiratory exchange ratio (RER), all monitored continuously throughout testing (40). Heart rate (HR, Polar FS2, Polar Electro, Inc, Lake Success, NY) was also continuously monitored throughout testing. Cadence (spm) and work rate (WR, watts (W) resistance) were monitored and recorded every 30 seconds.

Blood samples were obtained by finger or ear punctures immediately before testing, and every 1000m during testing, as well as 5- and 10- min post-testing. Blood lactate concentrations were determined by the Lactate Pro analyzer (FaCT Canada, Quesnel, British Columbia, Canada) (41), which we have concluded to be valid and accurate against an enzymatic assay ($r=0.64$, $p<0.001$, Sigma Diagnostics, St. Louis, MO), as is consistent with the peer-reviewed literature (42-45).

Subjects were instructed to not consume food or beverages other than water, or to perform any strenuous physical activities 2 h prior to testing. To control for the effects of dietary intake and hydration status, subjects were instructed to record all food and fluid intake 7d prior to testing, as well as the day of exercise testing (Appendix 7). Subjects had the opportunity to warm-up for at least 10 min prior to all testing.

Rowers performed two tests in the lab. The first was a pre-test done in order to acclimate subjects to testing protocol and laboratory procedures, as well as to establish a VO_2peak and target WR of 85% of their maximal work rate (WR_{max}) for the subsequent 4K TT. VO_2peak was determined by a modified version of the maximum aerobic power (MAP) test, which is a ramped protocol used by rowing coaches to assess training progress (46).

Rowers' MAP in split-time was converted into watts ($W = 2.8/\text{pace per } 500 \text{ m}^3$), and the test began 100 W below the predicted maximum. Each stage of the test lasted 90s, with a 10s "gear-up" period between each stage (Appendix 12). Every 90 s, the rower was asked to increase her WR by 20 W, until she was no longer able to maintain the WR. This test was designed to last between 8-10 min. VO_2peak was identified as the highest VO_2 value achieved, and was confirmed by at least one of the following: 1) VO_2 increased by $<150 \text{ ml/min}$ with an increase in WR; 2) $\text{RER} > 1.10$; or 3) HR_{max} was within 10 beats of age-predicted maximum ($220 - \text{age}$) (47). A 15-min cool-down period followed testing at a self-selected WR, and HR was monitored for 10 min post-test. Blood sampling for lactate was collected pre- and post test, as well as at 5- and 10-min post-test. Complete test time was about 45 min (10-15 min to acclimate to equipment; 10 min for actual testing; 15 min cool-down). Participants were able to stop the test at any time, and the investigator was able to stop the test at any time (equipment malfunction; subject symptoms of severe fatigue).

Endurance capacity was assessed by time to complete a 4K TT, and was administered within 3 days of the pre-test. This test consisted of a 4K ergometer row at a sub-maximal WR prescription (WR_{Rx}) of 85% of rowers' VO_2peak reached in the

pre-test. This WR was maintained for 3600m of the test, and the rowers were then asked to sprint the final 400m of the test to simulate on-water racing (Appendix 12). The 4K TT was designed to last about 20-25 min. Subjects received standardized verbal encouragement during testing. Complete testing time was ~ 60 min total (15 min to acclimate to equipment; 5 min warm-up; 25 min actual testing; 15 min cool-down). VO_2 peak and other performance outcome variables were obtained from the 4K TT.

Calculations:

Work output (kcal/min) was calculated as:

$$\text{Formula 5.1: Work output} = [\text{Work (W)} * 0.014 \text{ kcal/min}].$$

Energy expenditure (EE) input was calculated as:

$$\text{Formula 5.2: EE} = [\text{VO}_2 \text{ (L/min)} * \text{non-protein respiratory quotient (kcal/min)}].$$

Gross energetic efficiency (EF) was calculated as(48) :

$$\text{Formula 5.3: Gross EF} = [\text{Work output (kcal/min)} / \text{EE input (kcal/min)}] * 100$$

Deviation from the target WR_{Rx} of 85% of their pre-test WR_{max} (W) was calculated as:

$$\text{Formula 5.4: Deviation from } \text{WR}_{\text{Rx}} = [\text{WR}_{\text{Rx}} - \text{average W maintained}].$$

Data Analysis: All data were analyzed using SPSS Statistics version 18.0 (Chicago, IL). Descriptive statistics are presented as means \pm standard deviations (SD). Student's T-test and ANOVA was used to analyze differences between iron status groups (*Normal*: sFer \geq 20.0 μ g/L vs *Depleted*: sFer $<$ 20.0 μ g/L) with respect to all variables measured. Pearson's correlations were used to examine associations between variables. To investigate the plausible effects of iron status on endurance performance, multiple regression analyses (MIXED procedure with School as a random effect) of the relationship between measures of physical performance (VO₂ peak, gross EF, 4K TT time) and total body iron stores was conducted, controlling for potential confounders. Those variables correlated with physical performance ($p < 0.05$) or significantly different between groups of iron status were designated as potential confounders and included as covariates in the development of regression models. A statistical significance level of $p < 0.05$ was accepted for testing main effects, and $p < 0.10$ for testing interaction effects of the primary hypotheses.

Results

Body size and composition in the two iron status groups is shown in Table 5.1. There were no significant differences between groups for any measure of body size or body composition tested. On average, rowers were 19.8 \pm 1.1 years of age, 170.7 \pm 7.3 cm tall, and weighed 69.5 \pm 8.1kg (BMI 23.7 \pm 3.3 kg/m²).

Table 5.1: Anthropometry and body composition (mean±SD) of non-anemic female collegiate rowers at the beginning of training season, between groups*

	Normal (n=24)	Depleted (n=24)	p-value (T-test)
Weight (kg)	68.3±7.1	67.5±9.6	0.72
Height (cm)	170.5±5.9	170.2±8.9	0.88
Body mass index (kg/m ²)	23.5±2.1	23.3±2.7	0.80
Sitting height (cm)	86.8±5.7	87.0±10.7	0.93
Arm span (cm)	168.7±8.6	169.1±10.5	0.91
Percent body fat (%)	24.6±5.0	26.3±5.3	0.26
Fat-free mass (kg)	51.3±5.0	49.5±6.1	0.27

*Normal = sFer>20.0 µg/L, Hgb>12.0 g/dL; Deplete = sFer<20.0 µg/L, Hgb>12.0 g/dL

There were also no significant differences between the groups in any of the potential confounders of the association between iron status and performance that were tested (Table 5.2). Across the sample, rowers had 3.2±2.2 years rowing experience; a 2K PR time of 7 minutes and 48 seconds (471±28 sec); and spent ~5.5 (5.5±3.9 hours/week) hours per week in combined moderate and vigorous physical activity. There was, however, a marginal difference in rowers' reported 2K personal record from the previous season. The 24 rowers who were *Depleted* reported 2K PRs that were ~18 seconds slower than those 24 with *Normal* iron status (8.0±0.6 versus 7.7±0.4 min, respectively; p=0.07).

Table 5.2. Rowing experience and dietary characteristics (Mean \pm SD) of subjects

	<i>Normal</i> (n=24)	<i>Depleted</i> (n=24)	p-value
Age (y)	20.1 \pm 1.1	19.6 \pm 1.1	0.12
Rowing experience (y)	3.5 \pm 2.3	2.9 \pm 2.2	0.37
2K personal record (min)	7.7 \pm 0.4	8.0 \pm 0.6	0.07
Dietary iron intake (mg/d)*	16.3 \pm 5.9	16.3 \pm 7.1	0.99
Energy intake (kcal/d)*	1988.5 \pm 622.0	1759.2 \pm 526.3	0.26
Current use of supplement (%)	9/22 = 40.9%	8/23 = 34.8%	0.76
Number of days since first day of last menstrual period (d)	18.5 \pm 12.2	17.4 \pm 13.5	0.77

*n=15 in Normal, n=18 in Deplete groups.

There were no differences between the two iron status groups in dietary iron intake, energy intake, or current supplement use. Rowers who completed the 7d food diary reported consuming ~16 mg of dietary iron per day, 1900 kcal/d, and 38% of rowers in the study reported consuming a multivitamin/mineral supplement prior to the study.

Iron status of the *Normal* and *Depleted* rowers is shown in Table 5.3. There were significant differences between the two groups in sFer and TBI, which was expected due to classification of subjects based on a sFer cut-off of 20.0 μ g/L. One subject in the *Depleted* group had an AGP greater than the normal reference range (55-140 mg/dL). The two groups did not differ in sTfR, Hgb, or any other red cell indicators.

Table 5.3: Iron status (mean± SD) of non-anemic female collegiate rowers at the beginning of training season, between groups*

	<i>Normal</i> (n=24)	<i>Depleted</i> (n=24)	p-value
Hemoglobin (Hgb, g/dL)	13.1±0.7	13.0±0.7	0.720
Serum Ferritin (sFer, µg/L)	43.0±20.3	13.9±5.1	<0.001
Log sFer (µg/L)	1.1±0.2	1.6±0.2	<0.001
Soluble Transferrin Receptor (sTfR, mg/L)	6.4±2.5	6.4±2.1	0.958
Total Body Iron (TBI, mg/kg)	5.2±2.0	1.2±2.3	<0.001
Hematocrit (Hct, %)	40.1±2.1	40.2±2.1	0.90
Mean Cell Volume (MCV, fL)	88.8±3.6	87.9±4.6	0.34
Red Cell Distribution Width (RDW, %)	12.8±0.7	13.6±2.0	0.07
Alpha-1-acid glycoprotein (AGP, mg/dL)	79.6±23.3	79.4±33.0	0.98

**Normal* = sFer>20.0 µg/L, Hgb>12.0 g/dL; *Deplete* = sFer<20.0 µg/L, Hgb>12.0 g/dL

Results of the 4K ergometer test are shown in Table 5.4. There were no significant differences in maximal HR, WR, or average gross EF between the two groups. *Depleted* rowers had a lower absolute VO₂peak (p=0.03), and tended to have a lower VO₂peak/ kg FFM (p=0.10) than rowers with *Normal* iron status. *Depleted* rowers also tended to perform the fist 3600m of the 4K at a lower workload than the *Normal* rowers (p=0.07).

Table 5.4. Physical Performance (Mean±SD) over 4K at the beginning of a training season for female rowers with normal and depleted iron status

	Normal (n=24)	Depleted (n=24)	p-value, unadjusted for covariates*
Work rate Rx (W)	180.0±30.1	163.3±31.5	0.07
Deviation from Work rate Rx (W)	0.9±4.5	-0.4±3.8	0.28
Average Work rate for 3600m WR (W)	179.0±29.0	163.7±30.7	0.08
<hr/>			
4K TT time (min)	17.2±1.1	17.7±1.2	0.12
3600m time (min)	15.6±1.0	16.1±1.2	0.13
400m time (sec)	94.9±9.4	97.13±7.7	0.38
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VO ₂ peak (l/min)	3.4±0.4	3.1±0.4	0.03
VO ₂ peak (ml/kg/min)	49.5±5.6	46.6±5.6	0.08
VO ₂ peak (ml/kg FFM/min)	65.9±5.3	63.2±6.0	0.10
Maximal work rate (W)	236.7±41.4	225.0±44.4	0.35
Maximal heart rate (bpm)	188.6±14.3	191.08±9.4	0.49
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Average rate Energy expenditure (EE, kcal/min)	13.75±2.0	12.89±1.8	0.12
Total EE (kcal)	235.0±22.8	226.8±18.6	0.18
Average Gross energetic efficiency (%)	17.8±1.6	17.5±2.0	0.60

*ANOVA

Rowers with *Depleted* iron stores had significantly higher pre-test lactate concentration (p=0.02, Figure 5.2), as well as higher lactate concentrations at the 1000m and 2000m segments of the 4K TT (p=0.04 and p=0.02, respectively), and 10-

min post-test. There was no significant difference between the two groups in maximal lactate concentration achieved. When expressed as a percent of maximum lactate achieved, there were no significant differences between the two groups at any time point during the 4K TT.

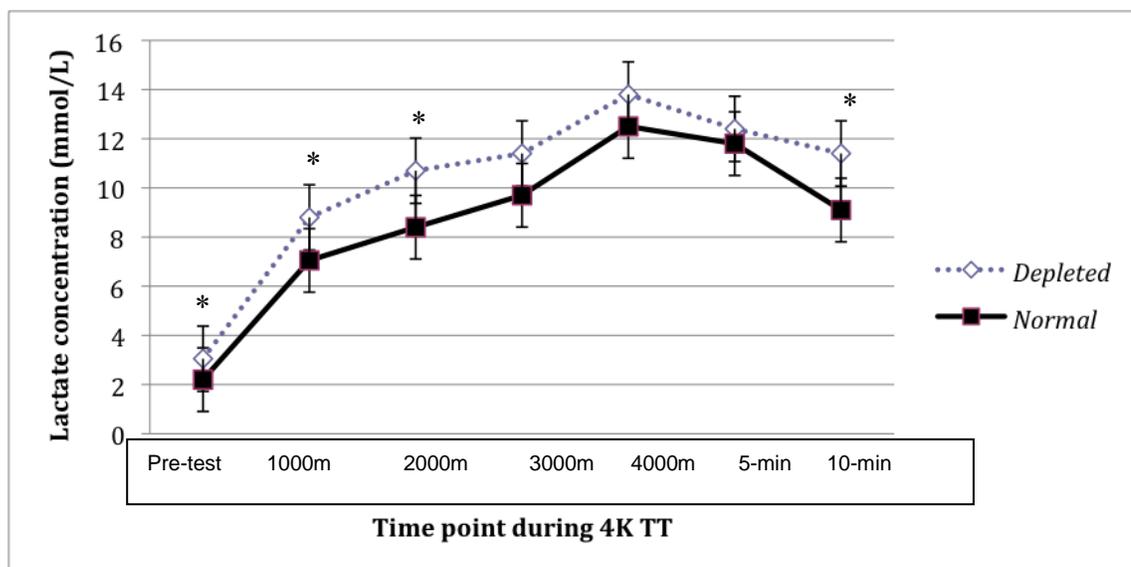


Figure 5.2. Lactate concentration (mmol/L) between Normal and Depleted rowers during 4K time trial (TT)

There was a significant difference in training session duration (Table 5.5).

Depleted rowers trained ~11 min/d less than Normal rowers (57.9 ± 9.2 versus 68.8 ± 21.0 min/d, respectively, $p=0.02$), and thus tended to have a lower average daily training load as calculated by session-RPE ($p=0.07$). There were no significant differences between the two groups in other variables measured over 7d.

Table 5.5. Training quality at the beginning of a training season

	Normal (n=24)	Depleted (n=24)	p-value
Sleep time (hrs)	6.8±0.7	7.2±1.0	0.16
Nap time (min)	64.0±34.9	64.3±78.3	0.99
Motivation	55.3±12.4	52.8±16.8	0.57
Soreness	35.6±15.5	31.2±15.3	0.33
Fatigue	45.4±14.8	39.5±13.8	0.16
Intensity	54.9±11.6	51.7±15.1	0.42
Total training days recorded	7.3±1.5	7.2±1.6	0.71
Average training time (min/d)	68.8±21.0	57.9±9.2	0.02
Average sessionRPE	3805.2 ±1570.8	3059.1±1207.3	0.07
Total training load	33508.0±18422.4	29222.9±19180.2	0.43

Simple bivariate correlations between iron status and 4K performance, and between training variables and 4K performance are shown in Tables 5.6 and 5.7, respectively. There was a significant positive correlation only between sFer and gross EF, but not between any other measures of iron status (Hgb, sTfR, TBI) and 4K performance. There were significant positive associations between body composition variables and 4K performance, but none between 4K performance and rowing experience, training time, or session RPE. Rowers' reported 2K personal records (PRs) were significantly related to the three outcome measures (VO₂peak, L/min: $r=-0.69$, $p<0.001$; 4K time, min: $r=+0.64$, $p<0.001$; gross EF, %: $r=-0.58$, $p<0.001$), validating this measure used in previous analyses (*Chapter 4*). Many of these

variables were tested as potential confounders, and no cause-effect relationships (or lack thereof) between training and performance are implied by these correlations.

Table 5.6: Bivariate correlations (r) between physical performance and iron status

	4K time (min)		VO ₂ peak (L/min)		Gross Energetic Efficiency (%)	
	r	p-value	r	p-value	r	p-value
Hemoglobin (g/dL)	-0.14	0.34	-0.08	0.58	+0.10	0.52
Serum Ferritin (µg/L)	-0.24	0.10	+0.29	0.05	+0.22	0.14
Log sFer (µg/L)	-0.25	0.09	+0.22	0.13	+0.25	0.09
Soluble transferrin receptor (mg/L)	-0.22	0.14	+0.23	0.12	+0.11	0.45
Total Body Iron (mg/kg)	-0.11	0.46	+0.10	0.49	+0.14	0.33

Table 5.7: Bivariate correlations (r) between physical performance and training

	4K time (min)		Gross Energetic Efficiency (%)		VO ₂ peak (L/min)	
	r	p-value	r	p-value	r	p-value
Height (cm)	-0.59	<0.001	+0.36	0.01	+0.58	<0.001
Weight (kg)	-0.49	<0.001	+0.30	0.04	+0.50	<0.001
Fat-free mass (kg)	-0.65	<0.001	+0.43	0.002	+0.71	<0.001
Rowing Experience (y)	-0.26	0.07	+0.15	0.30	+0.16	0.29
2K Personal Record (PR, sec)	+0.78	<0.001	-0.58	<0.001	-0.67	<0.001
Training time (min/day)	-0.12	0.42	-0.17	0.26	+0.12	0.42
SessionRPE	-0.04	0.78	-0.24	0.10	+0.06	0.69

ANOVA was then conducted for the main performance outcomes after adjusting for important covariates (Table 5.8). After adjustment, iron-depleted rowers completed the 4K TT 30 seconds slower, had a 0.3 L/min lower VO₂peak, and on average, were 0.6% less efficient compared to rowers with normal iron status.

*Table 5.8. Physical performance outcomes during 4K TT (mean ±SD), adjusted for covariates**

	Normal	Depleted	p-value
4K TT time (min)	17.43±0.67	17.92±0.87	0.030
VO ₂ peak (L/min)	3.38±0.33	3.08±0.38	0.005
Gross energetic efficiency (%)	17.71±1.00	17.14±1.02	0.057

**4K TT adjusted for training group, height, deviation from work load; VO₂peak adjusted for training group, fat-free mass; gross EF adjusted for training group, average WR for 3.6K*

In order to test for differences in performance between iron status groups, multiple regression analysis was performed to control for potential confounders. The results of this analysis are shown in Tables 5.9, 5.10, and 5.11. Only in the modeling of iron status on VO₂peak was sFer group (0=*Depleted*, 1=*Normal*, based on sFer cutoff of 20.0 µg/L) a significant predictor of performance. Rowers with *Depleted* iron stores had a VO₂peak that was 0.25 L/min lower than rowers with *Normal* iron stores (p<0.001, Table 5.9). sFer group alone was not a significant predictor of 4K time (p=0.12, Table 5.10) or gross EF (p=0.38, Table 5.11). Training group alone (0=Low, 1=High, based on the average daily training load session RPE cut-off of 3200) was not significantly related to VO₂peak (p=0.06), 4K time (p=0.26) or gross EF (p=0.73).

Table 5.9. Regression models to test the effects of iron status on VO₂peak (L/min)

	VO ₂ peak (L/min)		VO ₂ peak (L/min, with interaction)	
	β	p	β	p
Intercept	+0.26	0.49	+0.26	0.44
Serum ferritin Group (sFer group)	+0.25	<0.001	+0.42	<0.001
Training group*	+0.12	0.06	+0.27	0.003
sFer*Training group	-----	-----	-0.32	0.02
Fat-free mass (FFM, kg)	+0.05	<0.001	+0.05	<0.001
%Variance explained by Fixed effects				
Within School (residual) variance	75.0%		83.3%	
Between School variance	42.9%		57.1%	
% of total Variance between (due to) schools	36.4%		36.8%	

*Training group based on average daily load (0=<3200, 1=>3200)
sFer group: 0=Depleted (sFer<20.0 µg/L), 1=Normal (sFer>20.0 µg/L)

Table 5.10. Regression models to test the effects of iron status on 4K TT time (min)

	4K TT time (min)		4K TT time (min, with interaction)	
	β	p	β	p
Intercept	+29.8	<0.001	+29.7	<0.001
Serum ferritin Group (sFer group)	-0.48	0.12	-0.70	0.13
Training group*	-0.34	0.26	-0.55	0.19
sFer*Training group	-----	-----	+0.47	0.46
Height (cm)	-0.07	0.02	-0.07	0.02
Deviation from Work rate Rx (W)	-0.07	0.04	-0.07	0.04
%Variance explained by Fixed effects				
Within School (residual) variance	34.4%		30.1%	
Between School variance	46.6%		60.3%	
% of total Variance between (due to) schools	38.4%		66.7%	

*Training group based on average daily load (0=<3200, 1=>3200)
sFer group: 0=Depleted (sFer<20.0 µg/L), 1=Normal (sFer>20.0 µg/L)

Table 5.11. Regression models to test the effects of iron status on gross energetic efficiency (EF, %)

	Gross EF (%)		Gross EF (% with interaction)	
	β	p	β	p
Intercept	+11.5	<0.001	11.51	<0.001
Serum ferritin Group (sFer group)	-0.45	0.38	-1.35	0.075
Training group*	-0.17	0.73	-0.91	0.17
sFer*Training group	-----	-----	+1.68	0.094
Average work rate for 3.6 K (W)	+0.04	0.001	+0.04	<0.001
%Variance explained by Fixed effects				
Within School (residual) variance	29.6%		31.4%	
Between School variance	34.5%		75.9%	
% of total Variance between (due to) schools	53.9%		10.8%	

*Training group based on average daily load (0=<3200, 1=>3200)
sFer group: 0=Depleted (sFer<20.0 $\mu\text{g/L}$), 1=Normal (sFer>20.0 $\mu\text{g/L}$)

In order to test for the potential modifying effect of iron status on the relationship between training and performance, interaction terms for sFer group -by- training group were included in the multiple regression models (see Tables 5.9, 5.10, 5.11). This analysis revealed that training modified the relationship between iron status and performance such that each performance variable was more negatively affected by poor iron status in the “Low” trainers compared to the “High” trainers. While there was no significant interaction between sFer group and training group as it affected 4K TT time (p=0.46 for the interaction term, Figure 5.3), within the low-training group rowers with poor iron status took 42 seconds longer to complete the 4K TT compared to rowers with normal iron status (p=0.05).

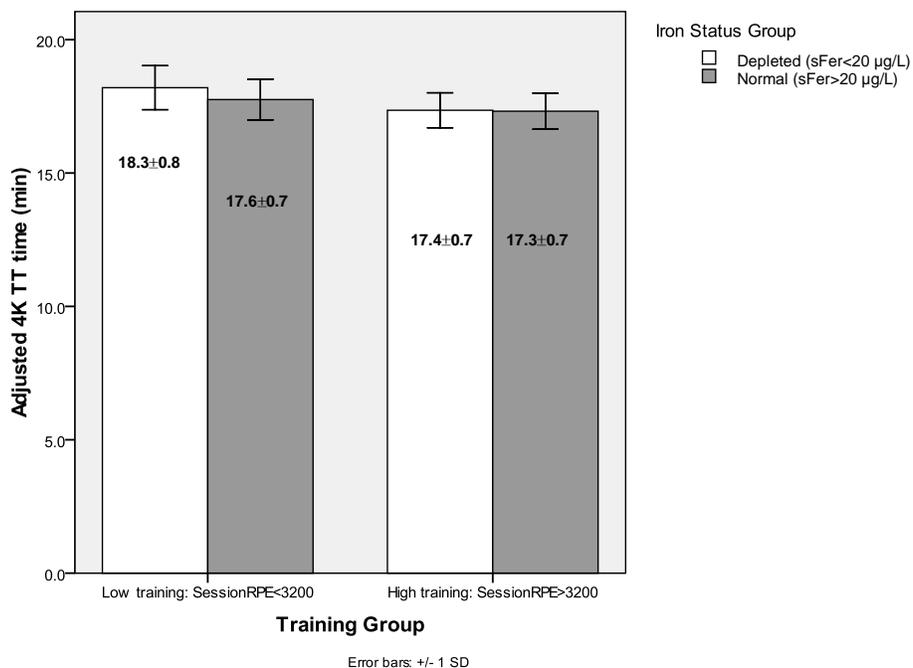


Figure 5.3. Time to complete 4K TT (min) for Low and High trainers according to iron status group

Additionally, less highly-trained rowers with poor iron status had VO_2 peak values that were 0.32 L/min lower (interaction $p=0.02$, Figure 5.4), and were 1.7% less energetically-efficient (interaction $p=0.094$, Figure 5.5) than less highly-trained rowers with normal iron status. Improved performance with improved iron status was only observed in the less highly-trained subjects.

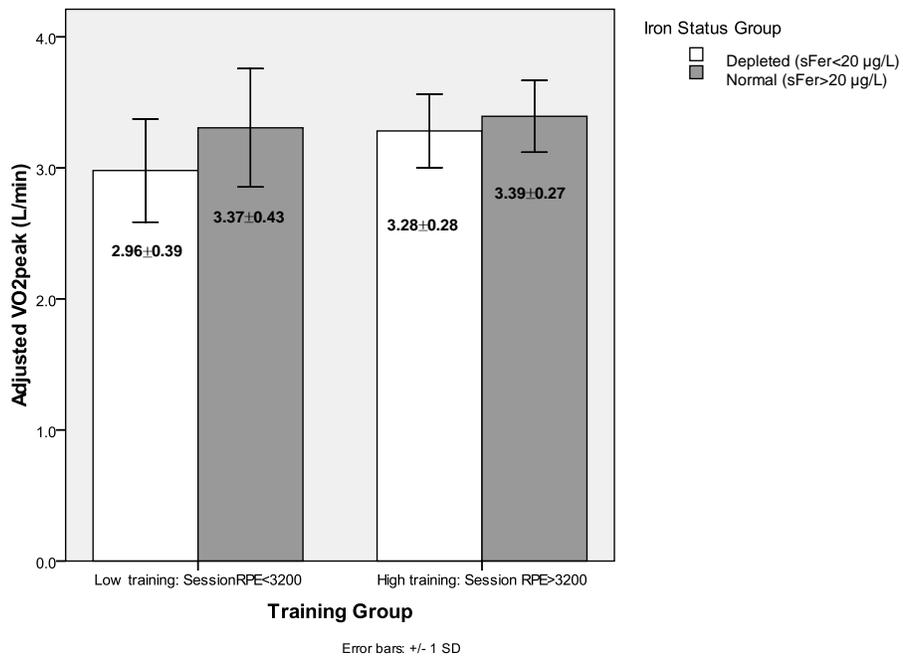


Figure 5.4. VO₂peak (L/min) for Low and High trainers according to iron status group

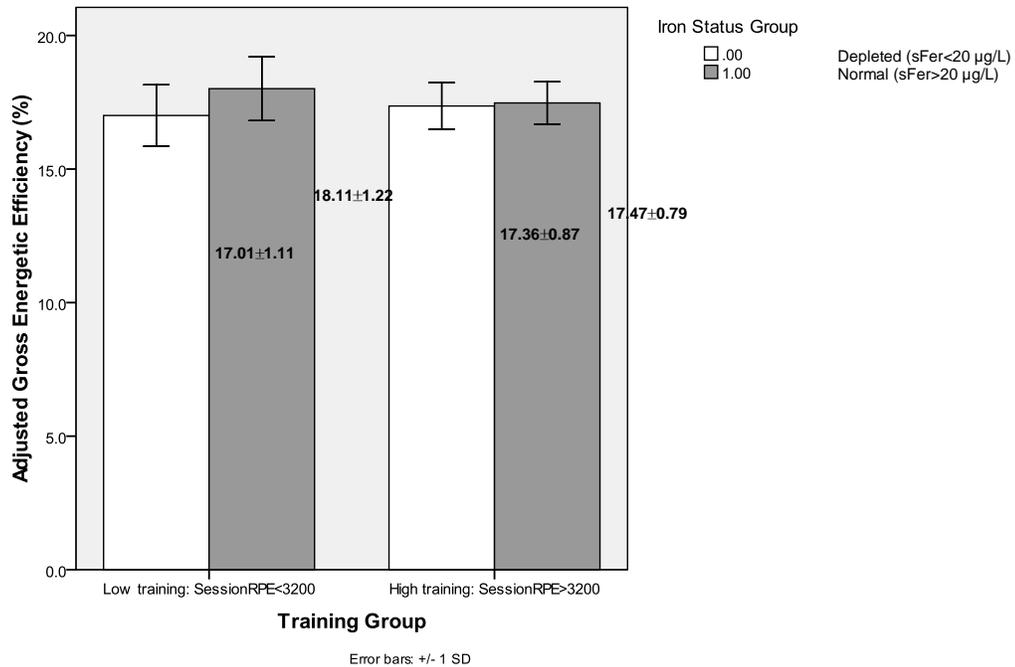


Figure 5.5. Gross energetic efficiency (%) for Low and High trainers according to iron status group

Discussion

These data support our hypothesis that rowers with *depleted* iron stores (sFer<20.0µg/L) have a lower VO₂peak and higher lactate concentration during the 4K TT compared to rowers with *normal* iron status. We have also observed that among women reporting lower levels of training time and intensity, time to complete a 4K TT was slower in iron-*depleted* rowers after controlling for training, height, and deviation from workload prescription, but there was not a significant interaction (p=0.46) between iron status group and training group. However, women in the low-training group completed the TT ~66 seconds (~30 sec adjusted for covariates) faster with normal iron status compared to those with depleted iron status. These findings are consistent with our lab's previous findings in non-athletes that showed that moderate training in previously untrained women with improved iron stores resulted in decreased 15 K TT time on a cycle ergometer by 10% (-3.4 min) compared to women with poorer iron status who decreased their time by 5% (-1.6 min) (16).

An important finding of this study was that rowers' training status differentially affected the relationship between iron status and performance (4K TT time, VO₂peak and gross EF). This finding could be interpreted in two ways. The first and more plausible interpretation is that rowers who trained less hard benefitted more from better sFer status than did rowers who trained harder. One can consider this a "ceiling effect," as there is less of a potential to benefit from better iron status in the more-highly trained. This could be why we did not see an effect of iron status on performance in the "High" training group. Our "Low" training group may be more comparable to the non-athletic women adapting to training, for whom there is a large

margin for improvement in performance (energetic efficiency and VO_2peak) with an improvement in iron status (12, 16). In the current study, less highly-trained rowers with *normal* iron status had a greater VO_2peak and were more energetically-efficient than those less highly-trained rowers with *depleted* iron stores, while there was no effect of iron status on performance in the more highly-trained group. This suggests that the effects of low iron status on VO_2peak can be overcome by training, as VO_2peak is an outcome related to O_2 transport at the end of the test. In non-anemic rowers, therefore, training would be a more important factor in the achievement of a higher VO_2peak compared to improved iron status. On the other hand, iron status may be more important than training level to improve energetic efficiency, as this outcome is related to metabolism over the entire TT, during which iron plays a significant metabolic role.

Another interpretation is that rowers that train harder are able to overcome the negative effects of poor iron status on performance. Animal studies have demonstrated that endurance training of ID rats seems to attenuate the reduction of skeletal muscle oxidative enzymes due to iron deficiency. In a study by Perkkio et al (49), although ID rats had poorer endurance performance, there was a significantly larger effect of endurance training in ID rats compared to non-ID rats. Although Hgb in this animal study was negatively affected by dietary iron deficiency, the activity of mitochondrial enzymes in the muscle of ID rats, as well as endurance capacity were partially remedied by endurance training. One possible explanation may be increased absorption of dietary iron in ID. As training increases and iron stores become depleted, the absorption of dietary iron may be up-regulated. Furthermore, it is likely that the

more highly-trained have already adapted more to the training regimen in spite of their iron status, while the less highly-trained still have potential to increase their training and therefore, their performance. Unmeasured behavioral and/or psychosocial characteristics of the rowers may explain this scenario, as prior training and rowing experience are important predictors of performance. It could be that rowers with more experience are more likely to train harder *despite* their iron status. If iron status affects the relationship between training and performance, it is only one of the many factors (physiological, psychological, environmental) to be considered.

Self-motivation is also an important variable, as it drives the training stimulus, and consequently performance. In a study of female college rowers, those who did not comply with the prescribed training regimens (e.g. comparable to our low training group) had lower self-motivation and poorer ergometer performance than those rowers who trained harder (e.g. comparable to our high training group) (50). In the current study, motivation scores were no different between the *Depleted* and *Normal* rowers, but more highly-trained rowers reported significantly higher motivation scores (58.5 ± 11.9) compared to less highly-trained rowers (49.6 ± 15.9 , $p=0.03$).

A limitation of this study is the possible misclassification of rowers into study groups of *Normal* and *Depleted* based on sFer cutoff of 20.0 $\mu\text{g/L}$. Any misclassification could have diluted the differences in physical performance between the two groups. Serum ferritin is the most common index of iron stores, and reflects iron stored in the liver. However, it is also an acute phase protein and can be elevated in an inflammatory state (e.g. infection, post-exercise), potentially masking ID (51-53). However, an inflammatory marker such as C-reactive protein (CRP) or alpha-1-

acid glycoprotein (AGP) can partially rule-out falsely-elevated sFer (54-56). In our study, while all but one subject had AGP levels below the threshold indicative of inflammation/infection, some iron-depleted subjects may still have been misclassified as normal.

Recent research has suggested that the iron regulatory protein hepcidin is the mediator between the inflammatory response and poor iron status (57). During the acute-phase response, which is seen post-exercise, hepcidin is up-regulated and enters the circulation to negatively control the export of iron from the intestinal enterocyte and recycled iron from the macrophage. Thus, increases in hepcidin may lead to a decrease in the absorption of dietary iron (9, 10, 58). Several studies have shown associations between or increases in urinary hepcidin and post-exercise inflammation, suggesting that high levels of training may negatively affect iron status (11, 59-64). This mechanism may explain the increased prevalence of iron deficiency in female athletes and/or changes in iron status with training. In the current study, hepcidin and iron absorption were not measured, while dietary iron intake was not significantly different between the two groups.

Soluble transferrin receptor (sTfR), a trans-membrane protein regulated by cellular iron status, reflects ID at the tissue level and is a more sensitive index of functional ID, especially at higher values (sTfR>8.5 mg/L). Unlike sFer, sTfR is unaffected by inflammation, and has been shown to have lower within-subject variability in intensely training athletes (3, 65). Total body iron (TBI) can be calculated using both sFer and sTfR (29). This index represents the ratio of functional to storage iron. However, in a population with a low prevalence of anemia, variation

in TBI is driven by variation in sFer. In the current study, only six rowers were in a state of severe iron deficiency (sTfR >8.5 mg/L), but were non-anemic, thus sTfR values were not significantly different between the *Depleted* and *Normal* rowers (mean sTfR 6.4 mg/L).

Additionally, protocols used to test the effects of iron status on performance are important to consider. The exercise protocol (eg. TT vs time to exhaustion) needs to be sensitive enough to detect a difference in performance due to iron status, as well as to adequately explore differences in energy metabolism. Previous studies of IDNA athletes have found no effect of iron status on performance during a time to exhaustion protocol (66, 67). The time to exhaustion protocol is not the best measure of athletic performance in endurance athletes for several reasons. The test becomes too long, especially for highly-trained athletes, and consequently motivation becomes a major factor affecting the outcome of the test (68). Furthermore, this protocol does not mimic competitive event performance. The 4K fixed length time trial (TT) was used in this study as a measure of endurance performance because it best represents rowers' training and competitive event performance (races last <30 min, short-duration, high-intensity). The 4K TT protocol also allows us to study proxies of oxidative metabolism (VO₂, energetic efficiency, lactate) throughout the test in which iron plays an important role, as mentioned previously (16).

Additionally, the measure of training needs to be sensitive enough to show difference between groups of iron status. In the current study, the session-RPE method was used to quantify training. Although this method has been used with various groups of athletes, and has been validated in our lab during rowing training, it

is possible that it inadequately captures the intermittent and varied nature of collegiate rowers' training activities. Much of rowing training revolves around repeated short bouts of high-intensity effort with intermittent rest (either on a rowing ergometer or in a boat on the water). It may have been more difficult for rowers to adequately gauge the intensity of an entire 2-hour workout if activity bouts were short with adequate rest between bouts of activity.

Conclusions: Despite the limitations of this cross-sectional study design, we found that in non-anemic rowers, iron depletion was associated with poorer physical performance at the beginning of a training season. The effects of iron status on performance were more pronounced in rowers who trained less hard compared to those who trained harder. Further studies that include more sensitive measurements of both training (e.g. HR and EE) and oxidative capacity (e.g. iron-dependent oxidative enzymes in muscle) are needed to better control for potential confounders and investigate the mechanism by which IDNA affects endurance performance. These results should be confirmed with a randomized controlled iron supplementation trial designed to establish causal pathways for the role of iron depletion on training and performance. Given the roles that iron plays in exercise, accurately determining iron status in female endurance athletes is critical. At the beginning of the training season, endurance athletes should be screened for iron deficiency with and without anemia, using both Hgb and sFer. Treatment and/or nutrition counseling should be provided as necessary to assure sufficient dietary iron intakes, and prevent further decrements in iron status with training.

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CHAPTER 6

IRON SUPPLEMENTATION IMPROVES IRON STATUS DURING TRAINING IN NON-ANEMIC FEMALE ROWERS

Abstract

Introduction: Studies in both animals and humans have shown a relationship between iron depletion without anemia (IDNA) and physical performance. Compared to their sedentary counterparts, female endurance athletes are at greater risk of IDNA.

Consequences of IDNA in non-athletic women are especially relevant to female endurance athletes, especially reduced work capacity, endurance, and energetic efficiency. We conducted a randomized placebo-controlled trial to investigate the effects of iron supplementation on iron status of female rowers during training.

Methods: At the beginning of a training season, 43 non-anemic collegiate female rowers were randomized to receive 100 mg/d ferrous sulfate (n=22) or placebo (n=21) for 6 weeks using a double-blind design. Thirty-one rowers (n=15 iron, 16 placebo) completed the 6-week trial and all baseline and endpoint testing. Subjects trained with their team as usual and completed daily training logs (time, type and intensity) and recorded compliance throughout the study. Iron status (hemoglobin, serum ferritin, soluble transferrin receptor, total body iron) was assessed at baseline and 6 weeks.

Results: At baseline, there were no significant differences between the two treatment groups in iron status. Rowers in both treatment groups increased their fat-free mass ($p < 0.001$) and improved their VO_2 peak ($p < 0.001$) after 6 weeks of training. Rowers in the iron supplemented group consumed 60% of the prescribed dose over the course of the study, and there was no difference in iron status between treatment groups at the

end of the study. Multiple regression analyses revealed improvements in body iron stores (log ferritin, total body iron) in the iron treatment group after controlling for baseline iron stores, and those with most depleted stores at baseline showed the greatest improvement in iron stores ($p=0.07$ for the treatment-by-baseline sFer interaction term).

Conclusion: Despite low compliance with the intervention and after controlling for baseline iron stores, iron supplementation improved iron stores in rowers training for their competitive season. We conclude that female endurance athletes who consume ~15 mg/d elemental iron during training can improve their iron stores, and those who are most depleted will benefit the most from supplementation. Furthermore, iron supplementation during training may prevent loss of iron status in those rowers beginning a competitive season with marginal, but adequate, iron stores.

Key words: *iron depletion, athletes, rowers, iron supplementation*

Iron supplementation improves iron status during training in non-anemic female rowers

Introduction

Iron deficiency (ID, clinically-defined as serum ferritin (sFer) <12.0 µg/L) is the most prevalent nutrient deficiency in the world, including the US, where iron deficiency with anemia (IDA, defined as ID plus hemoglobin (Hgb) <12.0 g/dL) affects 3-5%, and iron depletion without anemia (IDNA, defined as sFer <20.0 µg /L) affects ~16% of premenopausal women (1). Active females, including military soldiers, are especially susceptible, as surveys have shown even higher rates of IDNA (25-35%) (2-7). Although the exact mechanism is unknown, the increased prevalence of IDNA in active and training females may be due to increased daily iron requirements as a result of one or more factors, including: hemolysis (foot strike, impact); increased blood loss (gastrointestinal tract, hematuria, sweat); reduced iron absorption (8-11). These increased daily iron requirements of active and training women may not be met by dietary iron intake alone. Surveys of female athletes show that more than 50% use some type of supplement, and iron or iron-containing multivitamins are among the most popular (12-14).

It has been suggested from animal and human studies that iron status is related to physical training, but the direction of this relationship is unclear (15-21). Athletes' hematological adaptation to training has been reported, indicating increased mobilization of iron away from ferritin (storage) for the increased production of erythrocytes and increased demand for O₂-carrying capacity (Hgb) and functional tissue iron (soluble transferrin receptor, sTfR) (22, 23). Training sessions may also

cause an inflammatory response leading to increased hepcidin expression, and consequently decreased iron absorption and iron release from macrophages (24, 25). Additionally, researchers have also shown that sTfR is affected by muscle growth (26), which is a beneficial result of endurance training, and a factor that may play a role in diminishing the effects of supplementation on measures of iron status.

Previous work from our laboratory, as well as from others, has shown that iron supplementation of IDNA non-athletic women improves iron status, although results have varied due to dose used, as well as demand during training (27-31). Iron supplementation of female military soldiers has been shown to prevent declines in iron status that had been observed over 8 weeks of basic combat training (32).

Given the high prevalence of IDNA among female athletes, and the strong association between iron status and physical performance in both sedentary and active women, the current experimental study was designed to determine the efficacy of low-dose iron supplementation to prevent decrements in iron status in female rowers during 6 weeks of training for the competitive season. We hypothesized that iron supplementation would prevent training-related declines in iron status in female rowers, and that iron-supplemented rowers would improve their iron status after 6-weeks of treatment (no significant change in Hgb, increased sFer, decreased sTfR, increased TBI) compared to a placebo group.

Methods

Recruitment of subjects: Subjects were recruited at the beginning of the conditioning phases of their competitive collegiate rowing seasons (fall 2008, spring 2009, and fall 2009, upon arrival to campus post- summer and post-winter break). All

varsity and second-semester novice female rowers were eligible to participate in the screening if they were at least 18 years of age, non-smoking, and were able to begin regular training for their sport. Training activities during this time included general aerobic conditioning (cycling, running, rowing on ergometer), resistance training, and high intensity rowing. A medical screening required by the National Collegiate Athletic Association (NCAA) prior to our study excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc). All rowers provided written informed voluntary consent prior to participating in the study. This study was approved by the Institutional Review Boards of the following colleges/universities: Binghamton University, Cornell University, Hobart and William-Smith Colleges, Ithaca College, and Syracuse University.

A total of 165 female rowers from the five schools completed the iron status screening (Figure 3.2, pg 58). Ten percent of rowers (n=16) screened were identified as anemic and 30% as IDNA (sFer<20.0 µg/L, Hgb>12.0 g/dL). All 149 non-anemic subjects with and without iron depletion (cut-off: sFer≤20.0 µg/L) were invited to participate in the baseline physical performance and body composition testing (*Chapter 5*). Forty-eight rowers (n=24 IDNA rowers and 24 normal iron status) participated in the baseline data collection (n=5 William-Smith, 14 Cornell, 9 Ithaca, 12 Binghamton, and 8 Syracuse). Forty rowers were then randomized to receive supplemental iron (n=21, 12 with sFer≤20.0 µg/L) or placebo (n=19, 11 with sFer≤20.0 µg/L). Subjects received iron status, body composition, and fitness testing

results as a benefit of participation, along with referral and recommendations to improve iron status as necessary.

Design: This study was a randomized, double-blind, placebo-controlled iron supplementation trial (RCT). Each subject was randomly assigned to a treatment group by a research assistant who was not involved in data collection or contact with subjects. Randomization was done by assigning each subject a random number, with even and odd numbers being assigned to either treatment group. After initial randomization, any imbalance in the distribution of treatment or representation of school or baseline iron status (sFer) was corrected by re-randomization. Rowers were randomly assigned to one of two groups: iron supplementation with 50 mg FeSO₄ twice per day or placebo in the form of identical red capsules. Subjects were provided with 18 capsules each week, and were instructed to consume 2 capsules per day. Subjects were instructed to consume one capsule each at their morning and evening meals to minimize potential gastrointestinal side-effects, and with a glass of citrus juice to enhance iron absorption. Subjects were also instructed to avoid consumption of any other multivitamin/mineral supplements during the 6-week study period. At the time of randomization, no rowers had been regularly consuming dietary supplements.

Compliance with the iron treatment, as well as current health, menstrual status, and physical activity was assessed by daily training logs. Subjects were instructed to record the number of capsules they consumed daily in their log, even if they had consumed less than the prescribed amount per day. Additionally, weekly capsule counts were conducted by the researcher.

Both iron and placebo capsules were prepared by a Registered Pharmacist (PharmD) at the Cornell University College of Veterinary Medicine Pharmacy (Ithaca, NY). The iron supplement capsules contained 50 mg FeSO₄ per capsule with lactose filler, and the placebo capsules contained only lactose. The iron content of both placebo and iron capsules was analyzed via ICP mass spectrometry digestion by the USDA's Robert Holley Center for Agriculture and Health (Ithaca, NY). Twenty capsules were randomly selected for analysis from each of 2 batches. No differences in the average iron content were found between the two batches of iron-containing capsules (15.8 ± 0.5 mg elemental iron per capsule), and no iron was detected in the placebo capsules.

For all subjects, body composition and physical performance were measured immediately before and after the 6 week treatment period. Thirty-one rowers finished the entire study protocol (all blood analyses and exercise testing) (see Figure 3.2). Six subjects from the iron group and three from the placebo group dropped out of the study due to personal reasons (n=4), injury (n=3) or illness (n=2), all unrelated to the study. Rowers who did not complete the study reported getting more and better quality sleep at baseline ($p=0.01$ and $p=0.04$, respectively), as well as feeling better in general ($p=0.03$) than rowers who did complete the study (n=31). Compared to the 31 rowers who completed the study, the 9 rowers who did not complete the study had slower times to complete a simulated 4K time trial on a rowing ergometer at baseline (19.4 ± 2.6 min vs 17.7 ± 1.2 min, $p=0.008$). There were no other significant differences in baseline iron status, body composition, training, or performance measures between those who completed the study and those who did not complete the study.

Iron status variables measured from non-fasting venous blood samples (antecubital venipuncture, into two evacuated tubes, EDTA and serum-separator) included hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC, Beckman Coulter, Fullerton, CA); serum ferritin (sFer, Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL); soluble transferrin receptor (sTfR, Ramco Laboratories, Stafford, TX); alpha-1-acid glycoprotein (AGP, radial immunodiffusion plate, Kent Labs, Bellingham, WA). Total body iron (TBI, mg/kg) was calculated using the ratio of sTfR to sFer as described by Cook et al (33). Every effort was made to obtain baseline, midpoint, and endpoint samples at the same time of day to control for diurnal variation in any measurement of iron status. Immediately after blood sampling, Hgb and sFer status were analyzed. To control for potential variation in the non-automated sTfR assay conditions, both baseline and endpoint serum samples for the same subject were analyzed at the same time after the supplementation trial was completed. Rowers were classified as either iron depleted (sFer<20.0 µg/L), normal (sFer≥20.0 µg/L), or anemic (Hgb<12.0 g/dL). All anemics were notified of their status immediately after blood test results (within 1 week of analysis), referred to their respective campus health services for further instruction and/or monitoring, and excluded from further participation in the study. All laboratory assays were done in the Human Metabolic Research Unit at Cornell University (Ithaca, NY).

Body Composition: Anthropometric and body composition measurements were determined at the site of exercise testing. Body weight and height were measured with standard procedures and equipment (34). For athletes for whom it was accessible (n=31), body fat and fat-free mass was assessed via air-displacement plethysmography

(BodPod, Life Measurement, Inc, Concord, CA). For all subjects, percent body fat was calculated from tricep, suprailiac and thigh skinfold thickness (SF, Lange, Cambridge, MD) (35) and bioelectrical impedance analysis (BIA, RJL Systems, BIA-101) (36). The Siri equation (37) was used to calculate percent fat from body density. For those athletes without access to the BodPod (n=17), an average of their percent body fat values calculated from BIA and SF was used. In the sample with both BodPod and BIA-SF average (n=31), the two methods were highly correlated ($r=0.83$, $p<0.001$), and not significantly different from each other ($p=0.40$). The mean difference between percent body fat calculated from BodPod and the BIA-SF average was $-0.48\pm 3.15\%$ (95% confidence interval of difference: -1.64 , $+0.67$). There were no significant differences in either body fat measurement method across schools.

Data Analysis: All data were analyzed using SPSS Statistics version 18.0 (Chicago, IL). Based on an RCT of non-athletic women with good compliance, a sample size of 11 subjects per group was estimated to detect an effect size of 1.12 standard deviation units (reflecting a $6.41 \mu\text{g/L}$ difference in sFer between treatment groups at the end of the 6-week trial ($\alpha=0.05$, $\text{power}=0.80$) (30). We then used a smaller effect size (0.89) in order to account for the smaller margin for improvement in performance expected in athletes compared to untrained women (29, 30) due to their higher fitness level and training status at baseline, and estimated that a sample size of 17 rowers per treatment group should have been more than adequate to detect a difference in sFer between the two treatment groups.

Data were examined to verify normality of distribution, and skewed distributions were log-transformed. Results are reported as means \pm SDs. All initial

analyses were performed on an “as-treated” basis to examine the effect of iron supplementation on both iron status and performance outcomes. Additional subgroup analyses were then conducted on those subjects classified as IDNA at baseline.

Independent Student’s T-test and ANOVA was used to examine treatment group differences at baseline; characteristics differing between treatment groups ($p < 0.05$) were considered potential confounders and were included as covariates in subsequent regression models. Mixed linear regression analysis, including both random and fixed effects, was used to assess the effects of iron supplementation on change in iron status. School was treated as a random effect to control for unmeasured potentially confounding factors related to school differences in iron status. A statistical significance level of $p < 0.05$ was the level of statistical significance for main effects, and $p < 0.20$ for testing interaction effects.

Results

Subject characteristics: The placebo and supplemented groups were of similar age (19.8 ± 1.1 and 19.7 ± 0.9 years, respectively) and height (170.5 ± 7.7 and 169.0 ± 6.5 cm, respectively), and had similar years of rowing experience (3.7 ± 2.6 and 2.6 ± 1.3 years, respectively). There was no significant difference in history of supplement usage, and there was no difference in the number of days since last menstrual period at baseline between treatment groups (17.2 ± 10.2 and 22.9 ± 16.2 days in placebo and iron groups, respectively). Body weight and composition did not differ between the two groups before or after the study (Table 6.1). Rowers in both groups significantly increased their fat-free mass (FFM) by 1.2 ± 1.2 kg and decreased their percent body fat by 1.5 ± 1.8 % after 6 weeks of training ($p < 0.001$).

Table 6.1. Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (those who finished the trial)

	Pre-treatment	Post-treatment	Change
Weight (kg)			
Placebo (n=16)	67.7±9.5	67.8±9.4	0.09±1.2
Iron (n=15)	67.0±6.6	67.3±7.1	0.25±1.6
Body fat (%)			
Placebo	25.8±4.3	24.2±3.7*	-1.6±1.7
Iron	25.2±5.1	23.9±4.5*	-1.4±1.9
Fat-free mass (FFM, kg)			
Placebo	50.0±5.6	51.2±5.9*	1.2±1.1
Iron	50.0±4.9	51.1±5.1*	1.1±1.3

*Significantly different from baseline, $p < 0.001$

Compliance: There were no significant group differences in the number of recorded training days during the study period. The amount of capsules consumed, however, was 25% greater (but not significantly) in the placebo group (Table 6.2). There were no differences between the two groups in treatment-associated symptoms, as no adverse events or symptoms related to the supplementation were reported during the study.

Table 6.2. Compliance as measured by training log days recorded, weekly capsule count (n=31)

	Placebo	Iron
Training log days recorded, n	51±10	47±19
Capsules consumed, n	80±20	64±34*
Total FeSO ₄ consumed, mg	0	3200±1699
Percent of total prescribed dose consumed, %	75.6±17.7	60.3±30.2**

Between treatment groups: * $p = 0.11$, ** $p = 0.09$

Response to iron treatment: Results of the blood analyses measured at baseline and endpoint (0 and 6 weeks) of the study are presented in Table 6.3. No significant group differences in any iron status measure was observed at baseline. After 6 weeks of training and iron supplementation, intent-to-treat analysis revealed

there were no differences in iron status between the two treatment groups, although all major indicators of body/tissue iron stores changed in the predicted direction in the iron-supplemented group. Among those rowers with greater than 50% compliance (n=24), there were no significant differences in change in iron status between the two treatment groups. We did not find any significant correlations between the amount of iron supplement consumed and change in iron status in the iron treatment group.

Table 6.3. Iron status (Hgb, Hct, sFer, sTfR, etc) at baseline and endpoint of trial (n=31)

	Baseline (n=31)	Endpoint (n=31)	Change ¹
Hemoglobin, g/dL			
Placebo (n=16)	13.1±0.7	13.4±0.8	0.3±0.4
Iron (n=15)	13.1±0.7	13.3±0.6	0.2±0.7
Ferritin, µg/L			
Placebo	28.5±22.8	27.5±13.1	-1.0±14.8
Iron	25.0±15.8	28.0±8.6	3.0±15.3
Log sFer			
Placebo	1.3±0.3	1.4±0.2	+0.1±0.3
Iron	1.3±0.3	1.4±0.2	+0.1±0.3
Soluble transferrin receptor, mg/L			
Placebo	6.3±2.0	6.2±1.7	-0.1±2.2
Iron	6.2±1.9	5.4±1.7	-0.8±2.4
Total body iron, mg/kg			
Placebo	3.1±3.3	3.6±2.1	0.4±2.5
Iron	3.1±2.8	4.4±1.8	1.3±3.0

¹. Change=endpoints minus baseline

Initial multiple regression analyses (not including interaction terms) showed no effect of iron supplementation on change in iron stores after controlling for baseline iron stores (treatment group: $\beta = 0.04$ ($p=0.53$); $\beta = -0.78$ ($p=0.21$); $\beta = 0.85$ ($p=0.18$) for change in log sFer, sTfR, and TBI, respectively). After including the interaction term for treatment-by-baseline iron status, analyses showed that treatment group predicted

change in iron stores (as measured by log sFer and TBI) after controlling for baseline iron stores (Table 6.4).

Table 6.4. Regression models to test the effects of iron supplementation on change in iron stores (log sFer, sTfR, TBI) after 6 weeks of training and treatment (n=31)

	Model 1 – log sFer		Model 2 – sTfR		Model 3 - TBI	
	β	P	β	P	β	P
Constant	0.81	<0.001	4.69	0.003	2.29	0.001
Treatment (0=P, 1=I)	0.53	0.06	-0.13	0.95	1.77	0.06
Baseline log sFer ($\mu\text{g/L}$)	-0.56	<0.001	-----	-----	-----	-----
Baseline sTfR (mg/L)	-----	-----	-0.76	0.002	-----	-----
Baseline TBI (mg/kg)	-----	-----	-----	-----	-0.59	<0.001
Treatment*BL log sFer	-0.37	0.07	-----	-----	-----	-----
Treatment*BL sTfR	-----	-----	-0.10	0.75	-----	-----
Treatment*BL TBI	-----	-----	-----	-----	-0.30	0.18
R ² (adjusted R ²)	0.68 (0.65)		0.49 (0.43)		0.65 (0.61)	

The interaction between treatment group and iron status at baseline was significant, such that rowers with the lowest log sFer at baseline had the greatest improvement in iron stores in the iron group compared to the placebo group (p=0.07, see Figure 6.1). There was also a modest interaction effect of treatment-by-baseline TBI (p=0.18). Dose was not a significant predictor of change in sFer status in the entire sample of rowers, and was not included in the regression model. Furthermore, there was no significant interaction between treatment and dose as it affected change in stores ($\beta=0.003$, p=0.40).

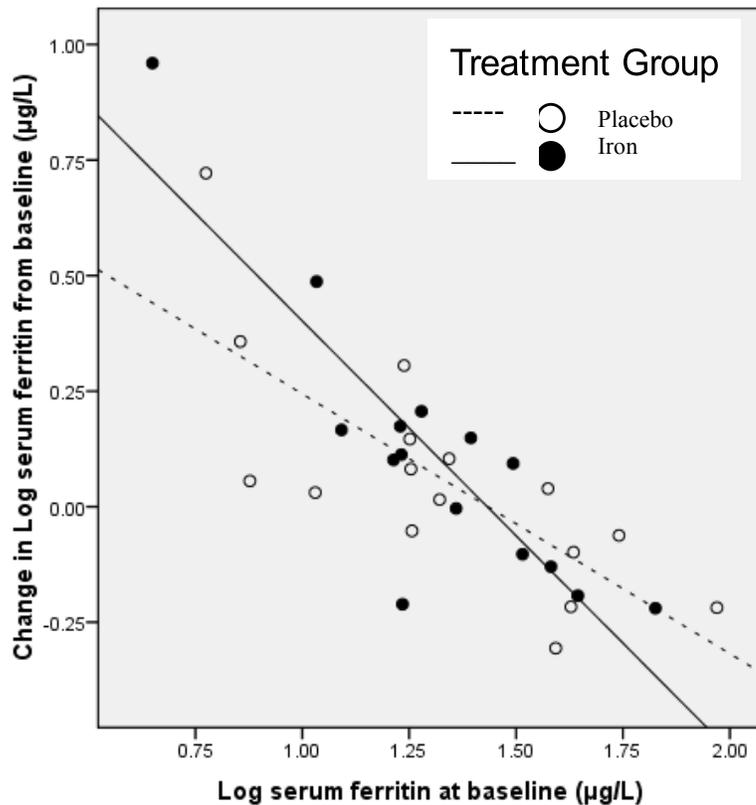


Figure 6.1. Relationship between log sFer at baseline and change in log sFer (Model 1 from Table 6.4) after 6 weeks of treatment (n=31). Interaction significant at $p=0.07$.

Additional plausibility analyses revealed a moderate relationship between supplemental iron consumed (mg elemental iron per kg of body weight) and change in total body iron (mg/kg) in the entire sample (n=14 iron, 16 placebo). After excluding one outlier who was a non-compliant rower in the iron treatment group with a large increase in TBI and controlling for TBI at baseline ($\beta=-0.58$, $p<0.001$), total supplemental iron consumed (0 mg in the placebo group) was a moderately significant predictor of change in TBI ($\beta=0.07$, $p=0.13$; $R^2=0.56$). Examining those rowers in the

iron group separately (n=14), total supplemental iron consumed remained a moderately significant predictor of change in TBI ($\beta=0.12$, $p=0.18$; $R^2=0.51$).

Subgroup analyses of rowers with IDNA at baseline

As a result of the significant interaction presented in Table 6.4 suggesting that the iron supplementation would be most beneficial to the rowers who were iron depleted at baseline, we separately examined the subgroup of rowers who started the study with sFer<20.0 µg/L. In these subjects, those randomized to the iron group were not significantly different from the placebo group in any baseline characteristics or measure of anthropometry or body composition (Table 6.5). Among this subgroup, there were no significant differences in compliance between the two treatment groups (Table 6.6).

Table 6.5. Anthropometry and body composition of rowers before and after 6 weeks of training and treatment

	Pre-treatment	Post-treatment	Change
Weight (kg)			
Placebo (n=8)	68.1±11.9	68.4±12.0	0.4±1.2
Iron (n=8)	65.3±6.0	65.1±6.2	-0.2±1.7
Body fat (%)			
Placebo	24.8±4.1	23.0±3.7	-1.8±1.9
Iron	26.7±5.2	24.8±4.3	-1.9±2.3
Fat-free mass (kg)			
Placebo	50.9±7.1	52.4±7.8	1.6±1.0
Iron	47.7±4.1	48.8±3.7	1.1±1.5

Table 6.6. Compliance as measured by training log days recorded, weekly pill count (n=16)

	Placebo (n=8)	Iron (n=8)
Training log days recorded, n	51.9±11.8	44.6±21.8
Capsules consumed, n	83.4±23.0	58.8±39.1
Total FeSO ₄ consumed, mg	0	2887.5±1871.0
Percent of total prescribed dose consumed, %	76.1±16.9	55.2±33.5

Response to iron treatment: Iron status of rowers with IDNA at baseline is presented in Table 6.7. After 6 weeks of training and iron supplementation, there were no differences in iron status between the two treatment groups, although all major indicators of body/tissue iron stores changed in the predicted direction in the iron-supplemented group. We did not find any significant correlations between the amount of iron supplement consumed and change in iron status in the iron treatment group.

Table 6.7. Iron status (Hgb, Hct, sFer, sTfR, etc) at baseline and endpoint of trial for rowers with IDNA at baseline (n=16)

	Baseline (n=16)	Endpoint (n=16)	Change
Hemoglobin, g/dL			
Placebo (n=8)	12.9±0.6	13.2±0.7	+0.3±0.2
Iron (n=8)	13.3±0.6	13.4±0.8	+0.2±0.8
Ferritin, µg/L			
Placebo	12.8±5.5	20.7±9.3	+7.9±9.4
Iron	14.3±4.8	25.1±9.5	+10.9±13.0
Log sFer			
Placebo	1.1±0.2	1.3±0.2	+0.2±0.3
Iron	1.1±0.2	1.4±0.2	+0.2±0.3
Soluble transferrin receptor, mg/L			
Placebo	6.0±1.8	6.6±1.0	+0.6±1.4
Iron	5.9±1.8	5.7±1.6	-0.2±2.3
Total body iron, mg/kg			
Placebo	1.1±2.6	2.3±1.7	+1.2±2.4
Iron	1.6±2.5	3.8±1.8	+2.1±3.5

Multiple regression analysis of the IDNA subgroup showed that baseline iron stores predicted change in iron status (as measured by log sFer, sTfR, and TBI, Tables 6.8, 6.9, 6.10), but there was no significant effect of treatment on change in iron status before including the treatment-by-dose interaction term. There was a significant

interaction between treatment group and dose consumed (Table 6.8, Model 1) for change in log sFer, and there were no significant interactions between treatment group and dose for the other measures of iron status.

Table 6.8. Regression models to test the effects of iron supplementation on change in log serum ferritin (log sFer) after 6 weeks of training and treatment in IDNA subgroup (n=16)

	Model 1 – log sFer		Model 2 – log sFer, without interaction		Model 3 – log sFer, without dose	
	β	P	β	P	β	P
Constant	1.85	0.002	1.30	0.002	1.38	0.001
Treatment (0=P, 1=I)	-0.44	0.26	0.13	0.29	0.10	0.34
Dose	-0.005	0.27	0.001	0.58	-----	-----
Log sFer at baseline	-1.17	0.001	-1.11	0.001	-1.10	0.001
Treatment*Dose	0.008	0.14	-----	-----	-----	-----
R ² (adjusted R ²)	0.67 (0.55)		0.59 (0.49)		0.59 (0.51)	

Table 6.9. Regression models to test the effects of iron supplementation on change in soluble transferrin receptor (sTfR) after 6 weeks of training and treatment in IDNA subgroup (n=16)

	Model 1 - sTfR		Model 2 – sTfR, without interaction		Model 3 – sTfR, without dose	
	β	P	β	P	β	P
Constant	5.21	0.07	4.15	0.02	5.36	0.001
Treatment (0=P, 1=I)	-1.89	0.47	-0.58	0.43	-0.92	0.19
Dose	0.002	0.95	0.017	0.23	-----	-----
Baseline sTfR	-0.79	0.002	-0.80	0.001	-0.79	0.002
Treatment*Dose	0.02	0.60	-----	-----	-----	-----
R ² (adjusted R ²)	0.63 (0.50)		0.62 (0.53)		0.57 (0.51)	

Table 6.10. Regression models to test the effects of iron supplementation on change in total body iron (TBI) after 6 weeks of training and treatment in IDNA subgroup (n=16)

	Model 1 - TBI		Model 2 – TBI, without interaction		Model 3 – TBI, without dose	
	β	P	β	P	β	P
Constant	5.49	0.12	2.29	0.18	2.26	0.005
Treatment (0=P, 1=I)	-2.33	0.52	1.42	0.19	1.43	0.14
Dose	-0.04	0.34	-0.0004	0.98	-----	-----
TBI Baseline	-0.99	<0.001	-0.96	<0.001	-0.96	<0.001
Treatment*Dose	0.05	0.29	-----	-----	-----	-----
R ² (adjusted R ²)	0.70 (0.60)		0.67 (0.59)		0.67 (0.62)	

The relationship between supplement dose and change in log sFer is shown in Figure 6.3 A and B. Iron-supplemented rowers who were more compliant (consumed more of the supplement) had the greatest improvement in iron stores (log sFer) compared to the placebo group ($\beta = +0.008$, $p=0.14$, see Figure 6.2A). These results persisted after excluding four subjects who did not consume at least 50% of the prescribed dose ($\beta = +0.013$, $p=0.15$ for the treatment-by-dose interaction, Figure 6.2B).

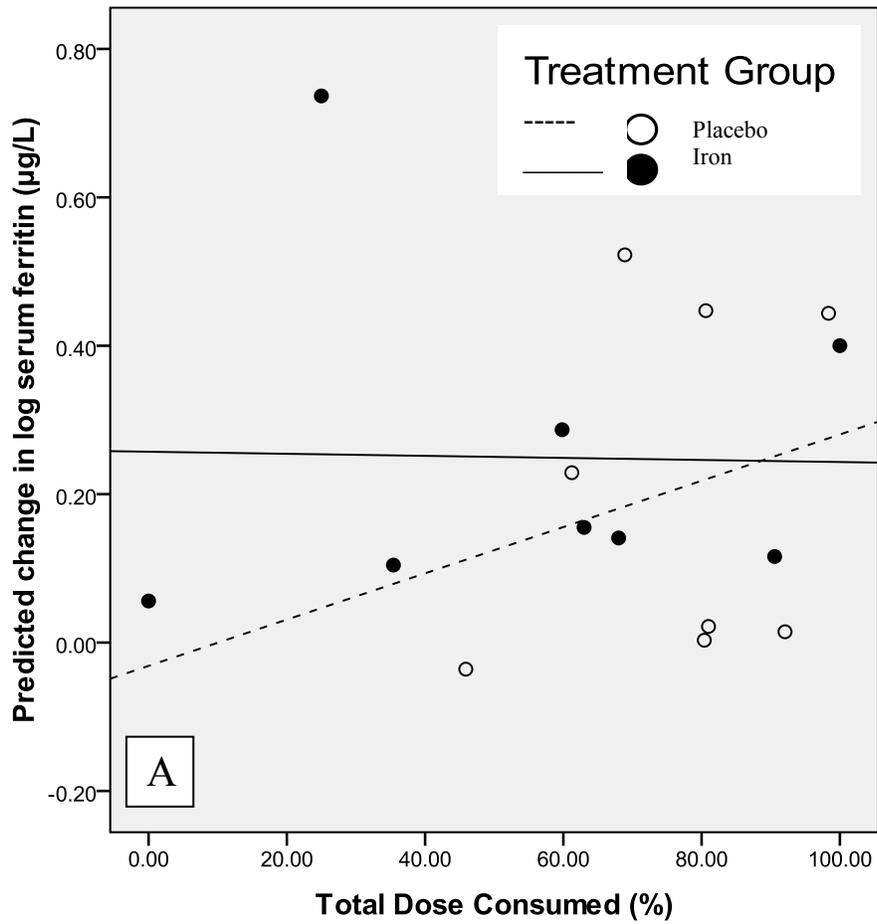


Figure 6.2 A. Relationship between supplement consumed (percent of total dose) and change in iron status (log sFer, Table 6.8, Model 1) after 6 weeks of treatment for all rowers IDNA at baseline ($n=16$;

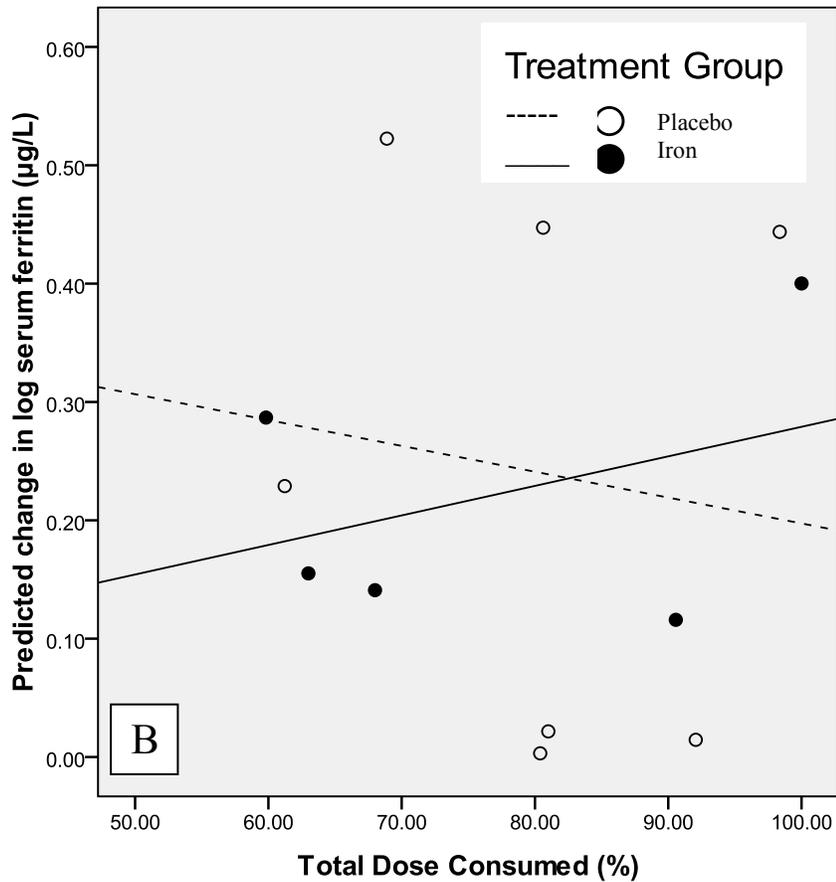


Figure 6.2B. Relationship between supplement consumed (percent of total dose) and change in iron status (log sFer, Table 6.8, Model 1) after 6 weeks of treatment for rowers IDNA at baseline consuming more than 50% of prescribed dose (n=12); $y=1.47 -0.92(\text{treatment group})-0.72 (\text{log sFer at baseline})-0.006(\text{total dose consumed}) +0.013 (\text{treatment*dose})$, $R^2=0.55$ (adjusted $R^2=0.29$)

Additional plausibility analyses revealed a moderate relationship between supplemental iron consumed (mg elemental iron per kg of body weight) and change in total body iron (mg/kg) in the IDNA subgroup sample (n=7 iron, 8 placebo). After excluding one outlier who was a non-compliant rower in the iron treatment group with a large increase in TBI, and controlling for TBI at baseline ($\beta=-0.68$, $p=0.002$), total supplemental iron consumed was a moderately significant predictor of change in TBI

($\beta=0.11$, $p=0.10$; $R^2=0.59$). Examining those IDNA rowers in the iron group separately ($n=6$), total supplemental iron consumed became a significant predictor of change in TBI ($\beta=0.23$, $p=0.05$; $R^2=0.79$).

Discussion

The purpose of this randomized, double-blind, placebo-controlled trial was to examine the effects of iron supplementation on change in iron status in non-anemic female rowers training for their competitive season. Serum ferritin and Hgb were used to identify those to include in the supplementation trial, as done previously (29, 30). Additionally, we measured sTfR and calculated total body iron to differentiate those with low total iron stores from those with low tissue (functional) iron (38-40). This study showed that 6 weeks of iron supplementation during training did lead to a greater improvement in iron stores (sFer, TBI) compared to placebo, after controlling for baseline iron stores. These findings are important to training female endurance athletes, as well as all physically-active women.

Other researchers have reported deterioration of iron status with moderate to high levels of physical activity. These decrements in iron status have been shown to affect physical performance, especially in those women who begin training with poor iron status (32, 40, 41), and iron supplementation has been shown to prevent a deterioration in sFer during periods of moderate to heavy physical training (32). In the present study, we did observe a positive effect of iron supplementation on sFer and TBI, especially in those rowers with low sFer at baseline. We did not, however, observe this positive effect on sTfR.

The rowers in our study were not anemic, therefore, there were few with sTfR >8.0 mg/L (n=6 at baseline). At the end of the study, sTfR was not significantly different between treatment groups (p=0.20), although rowers in the placebo group tended to have higher sTfR compared to the iron group. Researchers have shown that sTfR is affected by muscle growth (26, 40), and in the current study, after 6 weeks of training all rowers increased their FFM by ~1.2 kg. Although we observed no correlation between change in FFM and change in sTfR, it is possible that this factor may have played a role in diminishing the effects of supplementation on this measure of iron status.

In the current study, rowers with the lowest sFer at baseline (who had the greatest potential for improvement) did benefit the most from iron supplementation, which was expected. Depleted iron stores at the beginning of the training season, coupled with increased iron requirements during heavy training would result in negative iron balance. Increased intake of highly bio-available iron (dietary or supplemental) would be the only way to replace losses and meet increased demands of training (FFM, Hgb, etc). Conversely, if iron stores are adequate (or even marginally-adequate) at the beginning of a training season, they may be used to meet increased demands, thus leading to depletion over the course of a training period.

It is possible that rowers' training may have decreased (or overshadowed) the response to iron supplementation. Endurance exercise does increase body iron turnover, and may increase basal iron loss (sweat, urine, etc). It has been suggested that physical training may increase the estimated average requirement (EAR) for iron in female athletes by 30-70% -- from 8 mg to 10-14 mg/d (42). The level of iron

supplementation used in this study was more than the RDA for women (18 mg/d), and should have been adequate to improve iron stores if taken as directed. In previous studies, 100 mg FeSO₄ over the course of 6-8 weeks was sufficient to improve iron stores (sFer) in compliant, non-athletic women (29, 30). In our sample, although rowers in the iron group consumed on-average only 60% of the prescribed iron dose (~15 mg elemental iron/d), we still saw an improvement in iron status with iron supplementation, and no side-effects were reported with this dose.

Although women in this study were not clinically anemic, criteria used to identify anemia (Hgb <12.0 g/dL) may be insufficient for female athletes during training. At the end of the study, Hgb of rowers in both groups increased by 0.2 g/dL. The potential for functional (non-clinical) anemia is much greater in this population due to intense training, which may further increase demand for O₂-carrying capacity (Hgb) and functional tissue iron (sTfR). This prioritization of iron for Hgb synthesis over storage may be another reason for the small sFer response to supplementation.

A limitation of our subgroup analysis is the use of the sFer cut-off of 20.0 µg/L to identify rowers as IDNA. Any misclassification of baseline iron status in these subjects could have diluted the differences in change in iron status between the two treatment groups. Although sFer is the most common index of iron stores, and reflects iron stored in the liver, it is an acute-phase protein and can be elevated in an inflammatory state (e.g. infection, post-exercise), potentially masking ID (43-45). However, an inflammatory marker such as C-reactive protein (CRP) or alpha-1-acid glycoprotein (AGP) can partially rule-out falsely-elevated sFer (46-48). In our study, while there were no differences in AGP between the two treatment groups at the

beginning or end of the study, and only one subject had AGP levels above the threshold indicative of inflammation (140 mg/dL), some iron-depleted subjects may still have been misclassified as normal.

Recent research has suggested that the iron regulatory protein hepcidin is the mediator between the inflammatory response and poor iron status (49). During the acute-phase response, which is seen post-exercise, hepcidin is up-regulated and enters the circulation to negatively control the export of iron from the intestinal enterocyte. Thus, acute increases in hepcidin may lead to a decrease in the absorption of dietary iron (24). This mechanism may explain the increased prevalence of iron deficiency in female athletes and/or changes in iron status with training, as well as the reduced potential for improved sFer with iron supplementation in athletes. Several studies have shown associations between or increases in urinary hepcidin and inflammation post-exercise, suggesting that high levels of training may negatively affect iron status (11, 50-54). Researchers found that although serum hepcidin was not affected by basic combat training in female military soldiers, it was positively associated with sFer and CRP levels before and after training, demonstrating its association with inflammation (55). In the current study, hepcidin and iron absorption were not measured.

Conclusions: Despite our limitations, this randomized placebo-controlled study did show a significant effect of low-dose iron supplementation on change in sFer during training after controlling for baseline sFer, and adds to the growing body of evidence that iron supplementation improves the iron status of active women, which may ultimately impact training and physical performance. Based on the results from

this study, we conclude that female endurance athletes that consumed ~15 mg/d elemental iron during training can improve their iron status, and those who are most depleted will benefit the most from supplementation. Future studies should focus on ways to improve supplement compliance in training female athletes in order to confer maximum benefit to athletes' iron status, and to assess the effects of iron supplementation on training and physical performance in this population.

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CHAPTER 7

IRON SUPPLEMENTATION IMPROVES LACTATE RESPONSE AND ENERGETIC (WORK) EFFICIENCY AFTER TRAINING IN NON-ANEMIC FEMALE ROWERS

Abstract

Introduction: Studies in both animals and humans have shown a relationship between iron deficiency without anemia (IDNA) and physical performance. Twice as many active females (30%) are IDNA, compared to their sedentary counterparts.

Consequences of IDNA demonstrated in non-athletic women are especially relevant to female endurance athletes (e.g. reduced work capacity, endurance, and energetic efficiency). We conducted a trial to investigate the effects of IDNA on endurance performance in female rowers during training.

Methods: At the beginning of a training season, 43 non-anemic rowers were randomized to receive 100 mg/d FeSO₄ (n=22) or placebo (n=21) for 6 weeks using a double-blind design. Thirty-one rowers (n=15 iron, 16 placebo) completed the 6-week trial and all baseline and endpoint testing. Subjects trained with their team as usual and completed daily logs of training time, type and intensity throughout the study. Iron status (hemoglobin, serum ferritin, soluble transferrin receptor, total body iron), body composition, and laboratory tests of physical performance (4K time trial (TT), VO₂peak, energetic efficiency, blood lactate) were assessed at baseline and after training.

Results: There were no significant differences at baseline between the two treatment groups for iron status or performance. After controlling for baseline iron status,

multiple regression analyses revealed improvements in log sFer in the iron treatment group compared to placebo, and the more iron depleted at baseline, the greater benefit from supplementation. After 6 weeks of training, while rowers in both treatment groups increased their fat-free mass and improved their endurance performance (VO_2 peak, energetic efficiency, lactate response), lactate (as %lactate max) during the first 2000m of the TT and after 5 min of recovery was significantly lower in the iron-supplemented group compared to the placebo group ($p=0.05$). Multiple regression analyses revealed that after controlling for baseline performance and dose of supplement consumed, the rowers in the iron group who consumed more of the prescribed dose had a greater improvement in work efficiency compared to placebo ($p=0.15$ for the interaction treatment -by- dose). Additionally, the energetic efficiency of those rowers with poorer baseline iron stores ($sFer < 20.0$ ug/L) benefitted more from iron supplementation.

Conclusion: After 6 weeks of iron supplementation, rowers had improved lactate response during the first half of a 4K TT and 5 min post-test. After controlling for baseline performance and supplement dose, iron supplementation improved female rowers' energetic efficiency during a 4K TT after 6 weeks of training, but there was no effect on VO_2 peak or 4K TT time.

Key words: *iron depletion, athletes, physical performance, energetic efficiency, lactate, rowers, iron supplementation*

Introduction

Iron deficiency (ID, serum ferritin (sFer) <12.0 µg/L) is the most prevalent nutrient deficiency in the world, including the US, where iron deficiency with anemia (IDA, ID plus hemoglobin (Hgb) <12.0 g/dL) affects 3-5%, and iron deficiency without anemia (IDNA, sFer <20.0 µg/L) affects ~16% of young women (1). Active females, including military soldiers, are especially susceptible, as surveys have shown even higher rates of IDNA (25-35%) (2-7). Although research has clearly shown the effects of IDA on physical performance (8-10), the high prevalence of IDNA in female athletes requires investigation into its effects on physical performance in this group. Though the exact mechanism is unknown, the increased prevalence of IDNA in active and training females may be due to one or more factors beyond poor dietary iron intake, including: hemolysis (foot strike, impact); increased blood loss (gastrointestinal tract, hematuria, sweat); change in iron absorption (11-14). We have previously shown that daily iron supplementation for six weeks improves the iron status of female collegiate rowers (*Chapter 6*).

Despite the inability to identify the exact mechanism in humans, endurance capacity, energetic efficiency, and time trial (TT) performance have been shown to be impaired in laboratory studies of IDNA humans (15-18). Studies of how iron status affects non-anemic endurance athletes are limited, but research suggests that female endurance athletes and military soldiers with IDNA have impaired physical performance (19-24). It has been suggested that the iron status of experimental animals and humans is related to their physical training, but the direction of this relationship is unclear (8, 25-30).

Energetic efficiency and blood lactate metabolism are two measures of endurance performance. Energetic (work) efficiency (%) is defined as work output per kcal expended and is expressed as a percent relative to VO_2peak (ml/kg/min), or to work (W) performed. It is hypothesized that IDNA affects iron-containing enzymes of the TCA cycle, which are involved in the transformation of chemical to mechanical energy to do work (produce work output). Blood lactate concentration is positively correlated with the amount of lactate produced in muscle, which is an indication of the degree of anaerobic metabolism at the muscle tissue level. Blood lactate measured during exercise is the result of lactate production, release, and removal from the muscle. Although researchers have shown that ID and anemic humans and animals display an earlier increase in lactate production and decreased rate of lactate clearance (31, 32), results in IDNA humans are not clearly understood.

Previous work from our laboratory, as well as from others, has shown that IDNA reduces endurance capacity of non-athletes (both untrained women and untrained women participating in an aerobic training program) by improving energetic efficiency (15, 17, 18, 20). Additionally, our group and others have shown improvements in lactate metabolism in iron-supplemented women, especially during the earlier phases of endurance TTs (15, 17, 33). In a study of female athletes, we found that sFer status was a significant predictor of rowers' reported performance (2K Personal Record (PR) time, *Chapter 4*) and measured performance (VO_2peak , energetic efficiency, *Chapter 5*). Despite the strong cross-sectional associations between IDNA and performance, there is inadequate evidence demonstrating how iron

repletion affects performance in highly-trained IDNA endurance athletes, who have much less potential to benefit from improvements in iron status.

The high prevalence of IDNA among female athletes, and the strong association between both iron status and physical performance led us to perform the current study in which we aimed to test for a causal relationship between iron status and performance in trained female athletes who are beginning their competitive season's training program. The objectives of this study were to: 1) examine the effects of iron supplementation on female collegiate rowers' metabolic adaptations to rowing (endurance) training during a 4K TT (VO_2 peak, gross energetic efficiency, lactate concentration, and time to complete TT); and 2) to investigate the relation of change in iron status indicators and changes in endurance capacity and energy metabolism during a TT after 6 weeks of training. We hypothesized that iron-supplemented rowers would be more energetically-efficient and have improved blood lactate response during the 4K TT, complete the TT faster, and have a higher VO_2 peak than rowers in the placebo group.

Methods

Recruitment of subjects: Subjects were recruited at the beginning of the conditioning phases of their competitive rowing seasons (fall 2008, spring 2009, and fall 2009, upon arrival to campus post- summer and post-winter break). All varsity and second-semester novice female rowers were eligible to participate in the screening if at least 18 years of age, non-smoking, and were able to begin regular training for their sport. Training activities during this time included general aerobic conditioning (cycling, running, rowing on ergometer), resistance training, and high intensity

rowing. Detailed reports of training and physical activity will be reported elsewhere (*Chapter 8*). A medical screening (NCAA-required) prior to our study excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc). All rowers provided written informed voluntary consent prior to participating in the study. This study was approved by the Institutional Review Boards of the following colleges/universities: Binghamton University, Cornell University, Hobart and William-Smith Colleges, Ithaca College, and Syracuse University.

A total of 165 female rowers from the five schools completed the iron status screening (see Figure 3.2, pg 58). Ten percent of rowers (n=16) screened were identified as anemic and 30% as IDNA (sFer<20.0 µg/L, Hgb>12.0 g/dL). All 149 non-anemic subjects with and without iron depletion (cut-off: sFer≤20.0 µg/L) were invited to participate in the baseline physical performance and body composition testing. Forty-eight non-anemic rowers (n=24 IDNA and 24 rowers with normal iron status) participated in the baseline data collection (n=5 William-Smith, 14 Cornell, 9 Ithaca, 12 Binghamton, and 8 Syracuse). Forty rowers were then randomized to receive supplemental iron (n=21, 12 with sFer<20.0 µg/L) or placebo (n=19, 11 with sFer<20.0 µg/L). Subjects received iron status, body composition, and fitness testing results as a benefit of participation, along with referral and recommendations to improve iron status as necessary.

Design: This study was a randomized, double-blind, placebo-controlled iron supplementation trial. Each subject was randomly assigned to a treatment group by a research assistant who was not involved in data collection or contact with subjects.

Randomization was done by assigning each subject a random number, with even and odd numbers being assigned to either treatment group. After initial randomization, any imbalance in the distribution of treatment or representation of school or baseline iron status (sFer) was corrected by re-randomization. Rowers were randomly assigned to one of two groups: iron supplementation with 50 mg FeSO₄ twice per day or placebo in the form of identical red capsules. Subjects were provided with 18 capsules each week, and were instructed to consume 2 capsules per day. Subjects were instructed to consume one capsule each at their morning and evening meals to minimize potential gastrointestinal side-effects, and with a glass of citrus juice to enhance iron absorption. Subjects were also instructed to avoid consumption of any other multivitamin/mineral supplements during the 6 week study period.

Compliance with the iron treatment, as well as current health, menstrual status, and physical activity was assessed by daily training logs. Subjects were instructed to record the number of capsules they consumed daily in their log, even if they had consumed less than the prescribed amount per day. Additionally, weekly capsule counts were conducted by the researcher.

Both iron and placebo capsules were prepared by a Registered Pharmacist (PharmD) at the Cornell University College of Veterinary Medicine Pharmacy (Ithaca, NY). The iron supplement capsules contained 50 mg FeSO₄ per capsule with lactose filler, and the placebo capsules contained only lactose. The iron content of both placebo and iron capsules was analyzed via ICP mass spectrometry digestion by the USDA's Robert Holley Center for Agriculture and Health (Ithaca, NY). Twenty capsules were randomly selected for analysis from each of 2 batches. No differences

in the average iron content were found between the two batches of iron-containing capsules (15.8 ± 0.5 mg elemental iron per capsule), and no iron was detected in the placebo capsules.

For all subjects, body composition and physical performance were measured immediately before and after the 6 week treatment period. Thirty-one rowers finished the entire study protocol (all blood analyses and exercise testing) (see Figure 3.2). Six subjects from the iron group and three from the placebo group dropped out of the study due to personal reasons ($n=4$), injury ($n=3$) or illness ($n=2$), all unrelated to the study. Rowers who did not complete the study reported getting more and better quality sleep at baseline ($p=0.01$ and 0.04 , respectively), as well as feeling better in general ($p=0.03$) than rowers who did complete the study ($n=31$). Compared to the 31 rowers who completed the study, the 9 rowers who did not complete the study had slower times to complete a simulated 4K time trial on a rowing ergometer at baseline (19.4 ± 2.6 min vs 17.7 ± 1.2 min, $p=0.008$). There were no other significant differences in baseline iron status, body composition, training, or performance measures between those who completed the study and those who did not complete the study.

Assessment of iron status: Iron status variables measured from non-fasting venous blood samples (antecubital venipuncture, into two evacuated tubes, EDTA and serum-separator) included hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC, Beckman Coulter, Fullerton, CA); serum ferritin (sFer, Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL); soluble transferrin receptor (sTfR, Ramco Laboratories, Stafford, TX); alpha-1-acid glycoprotein (AGP, radial immunodiffusion plate, Kent Labs, Bellingham, WA). Total body iron (TBI, mg/kg)

was calculated using the ratio of sTfR to sFer as described by Cook et al (34). Every effort was made to obtain baseline, midpoint, and endpoint samples at the same time of day to control for diurnal variation in any measurement of iron status. Immediately after blood sampling, Hgb and sFer status were analyzed. To control for potential variation in the non-automated sTfR assay conditions, both baseline and endpoint serum samples for the same subject were analyzed at the same time after the supplementation trial was completed. Rowers were classified as either iron depleted (sFer < 20.0 µg/L), normal (sFer ≥ 20.0 µg/L), or anemic (Hgb < 12.0 g/dL). All anemics were notified of their status immediately after blood test results (within 1 week of analysis), referred to their respective campus health services for further instruction and/or monitoring, and excluded from further participation in the study. All laboratory assays were done in the Human Metabolic Research Unit at Cornell University (Ithaca, NY).

Body Composition: Anthropometric and body composition measurements were determined at the site of exercise testing. Body weight and height were measured with standard procedures and equipment (35). For athletes for whom it was accessible (n=31), body fat and fat-free mass was assessed via air-displacement plethysmography (BodPod, Life Measurement, Inc, Concord, CA). For all subjects, percent body fat was calculated from tricep, suprailiac and thigh skinfold thickness (SF, Lange, Cambridge, MD) (36) and bioelectrical impedance analysis (BIA, RJL Systems, BIA-101) (37). The Siri equation (38) was used to calculate percent fat from body density. For those athletes without access to the BodPod (n=17), an average of their percent body fat values calculated from BIA and SF was used. In the sample with both

BodPod and BIA-SF average (n=31), the two methods were highly correlated ($r=0.83$, $p<0.001$), and not significantly different from each other ($p=0.40$). The mean difference between percent body fat calculated from BodPod and the BIA-SF average was $-0.48\pm 3.15\%$ (95% confidence interval of difference: -1.64 , $+0.67$). There were no significant differences in either body fat measurement method across schools.

Questionnaires: Information about current dietary supplement use, health and menstrual status, usual physical activity, and eating habits and attitudes was obtained using questionnaires and a 7-day food diary. Rowing training regimen, as well as leisure-time physical activity (LTPA) outside of rowing training was quantified daily throughout the trial via detailed training and activity records (Appendix 11). Eleven questions in the daily log addressed sleep and nap duration and quality, soreness and fatigue, motivation, concentration, and training/physical activity frequency, intensity, time, and type. Questions were presented in the format of a Visual Analog Scale (VAS) (39). Subjects were asked to rate each question by placing a solid vertical line on a 100mm scale anchored by opposing descriptors. Data on training outcomes are presented elsewhere (*Chapter 8*).

The session-RPE method (40) was used to quantify daily training load. VAS Intensity score for each training session was multiplied by training session duration (time). This method has been used by others to quantify training in athletes (41-43). We validated the session RPE method using our VAS-format with the summated heart rate zone method (44) during two weeks of training on a separate sample of thirteen female rowers and found a significant positive correlation between the two methods ($r=0.85$, $p<0.001$, Appendix 1). In the current study, rowers were classified as either

“High” trainers or “Low” trainers based on the Session-RPE cutoff of 3200, which was the median (50th percentile) sessionRPE of the sample.

Physical Performance Testing Methods: Physical fitness and endurance performance was assessed using a rowing ergometer (Concept2, Morrisville, VT) equipped with a digital readout monitor (PM2), displaying work (W), stroke rating (SPM), distance (m), and time (min:sec). A computerized metabolic cart (TrueMax 2400, ParvoMedics, Salt Lake City, Utah) was used to measure VO₂ and other physiological measures during all testing. Concentrations of O₂ and CO₂ in expired air were analyzed with each breath, and respiratory volume (V_E) was measured with a respiratory pneumotachograph (Fitness Instrument Technologies, Farmingdale, NY) through a two-way breathing valve (Hans Rudolph, Kansas City, MO).

Energy expenditure was assessed via indirect calorimetry during exercise testing using a standard protocol that monitors expired gases for V_E, VO₂, VCO₂, and respiratory exchange ratio (RER) continuously throughout testing (45, 46). Heart rate (HR, Polar FS2, Polar Electro, Inc, Lake Success, NY) was also continuously monitored throughout testing. Cadence (strokes per minute, spm) and work rate (WR, watts (W) resistance) were monitored and recorded every 30 seconds.

Capillary blood samples were obtained by finger or ear punctures immediately before testing, and every 1000m during testing, as well as 5- and 10- min post-testing. Blood lactate concentrations were determined by the Lactate Pro analyzer (FaCT Canada, Quesnel, British Columbia, Canada) (47), which we have concluded to be valid and accurate against an enzymatic assay ($r=0.64$, $p<0.001$, Sigma Diagnostics, St. Louis, MO), as is consistent with the peer-reviewed literature (48-51).

Subjects were instructed to not consume food or beverages other than water, or to perform any strenuous physical activities 2 h prior to testing. To control for the effects of dietary intake and hydration status, subjects were instructed to record all food and fluid intake 7d prior to testing, as well as the day of exercise testing. Subjects had the opportunity to warm-up for at least 10 min prior to all testing.

Rowers performed two tests in the lab. The first was a pre-test done to acclimate subjects to testing protocol and laboratory procedures, as well as to establish a VO_2peak and target WR prescription of 85% of their maximal work rate (WR_{max}) to be used in the subsequent 4K TT. VO_2peak was determined by a modified version of the maximum aerobic power (MAP) test, which is a ramped protocol used by rowing coaches to assess training progress (52).

Rowers' MAP in split-time was converted into watts ($W = 2.8/\text{pace per } 500 \text{ m}^3$), and the test began 100 W below the predicted maximum. Each stage of the test lasted 90s, with a 10s "gear-up" period between each stage (Appendix 12). Every 90 s, the rower was asked to increase her WR by 20 W, until she was no longer able to maintain the WR. This test was designed to last between 8-10 min. VO_2peak was identified as the highest VO_2 value achieved, and was confirmed by at least one of the following: 1) VO_2 increased by $<150 \text{ ml/min}$ with an increase in WR; 2) $\text{RER} > 1.10$; or 3) HR_{max} was within 10 beats of age-predicted maximum ($220 - \text{age}$) (46). A 15-min cool-down period followed testing at a self-selected WR, and HR was monitored for 10 min post-test. Blood sampling for lactate was collected pre- and post test, as well as at 5- and 10-min post-test. Complete test time was about 45 min (10-15 min to acclimate to equipment; 10 min for actual testing; 15 min cool-down). Participants

were able to stop the test at any time, and the investigator was able to stop the test at any time (equipment malfunction; subject symptoms of severe fatigue). The pre-test was only performed at baseline.

Endurance capacity was assessed at both baseline and endpoint by time to complete a 4K TT, which was administered within 3 days of the baseline pre-test. This test consisted of a 4K ergometer row at a sub-maximal WR prescription (WR_{Rx}) of 85% of rowers' VO_2 peak reached in the pre-test. This WR was maintained for 3600m of the test, and the rowers were then asked to sprint the final 400m of the test to simulate on-water racing (Appendix 10.12). The 4K TT was designed to last about 20-25 min. Subjects received standardized verbal encouragement during testing. Complete testing time was ~ 60 min total (15 min to acclimate to equipment; 5 min warm-up; 25 min actual testing; 15 min cool-down). The 4K TT was performed at baseline and endpoint at the same WR Rx. VO_2 peak and other reported performance outcome variables were obtained from the 4K TT.

Calculations

Work output (kcal/min) was calculated as:

$$\text{Formula 7.1: Work output} = [\text{Work in Watts (W)} * 0.014 \text{ kcal/min}].$$

Energy expenditure (EE) input was calculated as:

$$\text{Formula 7.2: EE} = [\text{VO}_2 \text{ (L/min)} * \text{non-protein respiratory quotient (kcal/min)}].$$

Gross energetic efficiency (EF) was calculated as(53) :

$$\text{Formula 7.3: Gross EF} = [\text{Work output (kcal/min)} / \text{EE input (kcal/min)}] * 100$$

Deviation from the target WR_{Rx} of 85% of their pre-test WR_{max} (W) was calculated as:

$$\text{Formula 7.4: Deviation from } WR_{Rx} = [WR_{Rx} - \text{average W maintained}].$$

Data Analysis: All data were analyzed using SPSS Statistics version 18.0 (Chicago, IL). A sample size of 26 subjects per group was estimated as required to detect an effect size of 0.70 SD units for change in TT time and 29 subjects per group to detect an effect size of 0.65 SD units for change in VO_2 max between treatment groups ($\alpha=0.05$, $\text{power}=0.80$). Data were examined to verify normality of distribution, and skewed distributions were log-transformed. Results are reported as means \pm SDs. All initial analyses were performed on an “as-treated” basis to examine the effect of iron supplementation on both iron status and performance outcomes. Additional subgroup analyses were then conducted on those subjects classified as IDNA at baseline.

Independent Student’s T-test and ANOVA were used to examine treatment group differences at baseline; characteristics differing between treatment groups ($p<0.05$) were considered potential confounders and were included as covariates in subsequent regression models. Those variables significantly correlated with physical performance outcomes ($p<0.05$) were designated as potential confounders and included as covariates in the regression models. Pearson’s correlations were used to

examine the relation between change in iron status and change in performance outcomes.

Mixed effects linear regression analysis, including both random and fixed effects, was used to assess the effects of iron supplementation on performance. School was treated as a random effect to control for unmeasured potentially confounding factors related to school differences in iron status, training and performance. A statistical significance level of $p < 0.05$ was the level of statistical significance for main effects, and $p < 0.20$ for exploratory testing of interaction effects.

Results

Subject characteristics: Thirty-one rowers completed the performance testing at the end of the supplementation trial (see Figure 3.2). The placebo and supplemented groups were of similar age (19.8 ± 1.1 and 19.7 ± 0.9 years, respectively) and height (170.5 ± 7.7 and 169.0 ± 6.5 cm, respectively), and had similar years of rowing experience (3.7 ± 2.6 and 2.6 ± 1.3 years, respectively). Body weight and composition did not differ between the two groups before or after the study (Table 7.1). Rowers in both groups significantly increased their FFM by 1.2 ± 1.2 kg and decreased their percent body fat by 1.5 ± 1.8 % after 6 weeks of training ($p < 0.001$).

Table 7.1. Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (n=31)

	Pre-treatment	Post-treatment	Change
Weight (kg)			
Placebo (n=16)	67.7±9.5	67.8±9.4	0.09±1.2
Iron (N=15)	67.0±6.6	67.3±7.1	0.25±1.6
Body fat (%)			
Placebo	25.8±4.3	24.2±3.7	-1.6±1.7
Iron	25.2±5.1	23.9±4.5	-1.4±1.9
Fat-free mass (kg)			
Placebo	50.0±5.6	51.2±5.9	1.2±1.1
Iron	50.0±4.9	51.1±5.1	1.1±1.3

Differences between treatment groups not significant.

Compliance: There were no significant group differences in the number of recorded training days (51±10 and 47±19 d in Placebo and Iron groups, respectively) during the study period. The amount of supplement consumed, however, was 25% greater (not statistically significant) in the placebo group (Table 7.2). There were no differences between the two groups in treatment-associated symptoms (no adverse events or symptoms related to the supplementation were reported).

Table 7.2. Compliance as measured by training log days recorded, weekly pill count

	Placebo	Iron
Training log days recorded, n	51±10	47±19
Capsules consumed, n	80±20	64±34
Total FeSO ₄ consumed, mg	0	3200±1699
Percent of total Rx consumed, %	75.6±17.7	60.3±30.2

Response to iron treatment: Results of the blood analyses have been reported elsewhere (Chapter 6). Multiple regression analyses revealed improvements in body iron stores (log sFer, total body iron) in the iron treatment group after controlling for

baseline iron status, and those with most depleted stores at baseline showed the greatest improvement in iron status.

4K endurance time trial (TT): Results of the 4K TT before and after 6-weeks of training and treatment are shown in Table 7.3. After 6 weeks of training, rowers in both treatment groups improved VO_2peak ($+0.2\pm 0.2$ l/min, $p<0.001$) and maximal workload ($+22.0\pm 36.3$ W, $p=0.002$), however, there were no significant differences between the two treatment groups. While average gross efficiency during the entire 4K TT was not significantly different after 6 weeks of training in either treatment group ($p=0.39$), efficiency at the end of the test was significantly improved in both groups ($+1.2\pm 2.4\%$, $p=0.006$). There were no significant correlations between measures of iron status or change in iron status and change in performance in the total sample. As expected, there were significant positive correlations between change in performance and training variables in the total sample, which will be presented in *Chapter 8*.

Table 7.3. Physical performance measures (4K time, Total VO₂, % VO₂, RER, WR) before and after 6 weeks of training and treatment

	Pre-treatment	Post-treatment	Change
4K time, min			
Placebo (n=16)	18.0±1.0	17.7±1.4	-0.2±0.9
Iron (n=15)	17.4±1.3	17.8±1.9	0.4±1.3
Absolute VO ₂ peak, L/min			
Placebo	3.1±0.4	3.3±0.4*	0.2±0.2
Iron	3.3±0.4	3.4±0.4	0.2±0.2
Relative VO ₂ peak, ml/kg FFM/min			
Placebo	62.9±5.1	65.1±6.2	2.3±4.5
Iron	65.2±5.7	66.5±5.4	1.3±5.0
Maximal work rate, W			
Placebo	225.8±51.1	243.1±64.6**	17.3±35.0
Iron	227.3±25.9	254.3±43.7	27.0±38.2
Maximal heart rate, bpm			
Placebo	190.8±8.9	190.8±9.8	0.1±4.7
Iron	192.8±14.7	195.8±7.8	3.0±15.6
Maximal RER			
Placebo	1.00±0.04	1.04±0.07	0.04±0.08
Iron	1.03±0.07	1.05±0.07	0.02±0.07
Gross energetic efficiency, %			
Placebo	17.3±2.0	17.1±1.9	-0.2±1.3
Iron	16.9±1.1	17.5±1.3	0.6±1.2

No significant differences between treatment groups; *t*-test significantly different from BL, **p*<0.001, ***p*=0.002

Change in gross energetic efficiency: The effect of iron supplementation on change in performance as measured by VO₂peak, 4K TT time, and energetic efficiency was tested using multiple regression analyses after controlling for baseline performance, training, and supplement dose consumed. There were no significant effects of iron supplementation on 4K TT time or VO₂peak, but there were relationships between supplementation and gross energetic efficiency. Results of these analyses testing the effects of treatment group on energetic efficiency are shown in Table 7.4.

Table 7.4. Regression models to test the effects of iron supplementation on change in gross efficiency (%) after 6 weeks of training and treatment in entire sample of rowers (n=31)

	Model 1		Model 2		Model 3		Model 4 (n=24 consuming \leq 50% of prescribed dose)	
	β	p	β	p	β	p	β	p
Constant	3.15	0.14	6.3	0.01	7.9	0.005	7.6	0.02
Treatment (0=Placebo, 1=Iron)	0.37	0.33	0.02	0.97	-1.7	0.17	-3.90	0.17
Dose	-----	-----	- 0.02	0.04	- 0.04	0.02	-0.04	0.07
Baseline EF	- 0.22	0.08	-0.3	0.012	-0.3	0.008	-0.28	0.09
Training group (0=Low, 1=High)	1.32	0.002	1.5	<0.001	1.5	<0.001	1.6	0.003
Treatment*Dose	-----	-----	-----	-----	0.02	0.15	0.05	0.15
	R ² =0.45 Adj=0.38		R ² =0.53 Adj=0.46		R ² =0.57 Adj=0.49		R ² =0.56 Adj=0.44	

In the three regression models, while training and dose of supplement were significant predictors of change in efficiency, iron treatment alone was not (Table 7.4, Models 1 and 2, $p > 0.05$). There was a significant interaction between treatment group and dose consumed (Table 7.4, Model 3, $p = 0.15$). The more supplement that was consumed in the Iron group, the greater positive change in gross efficiency after 6 weeks of training and treatment (see Figure 7.1, B).

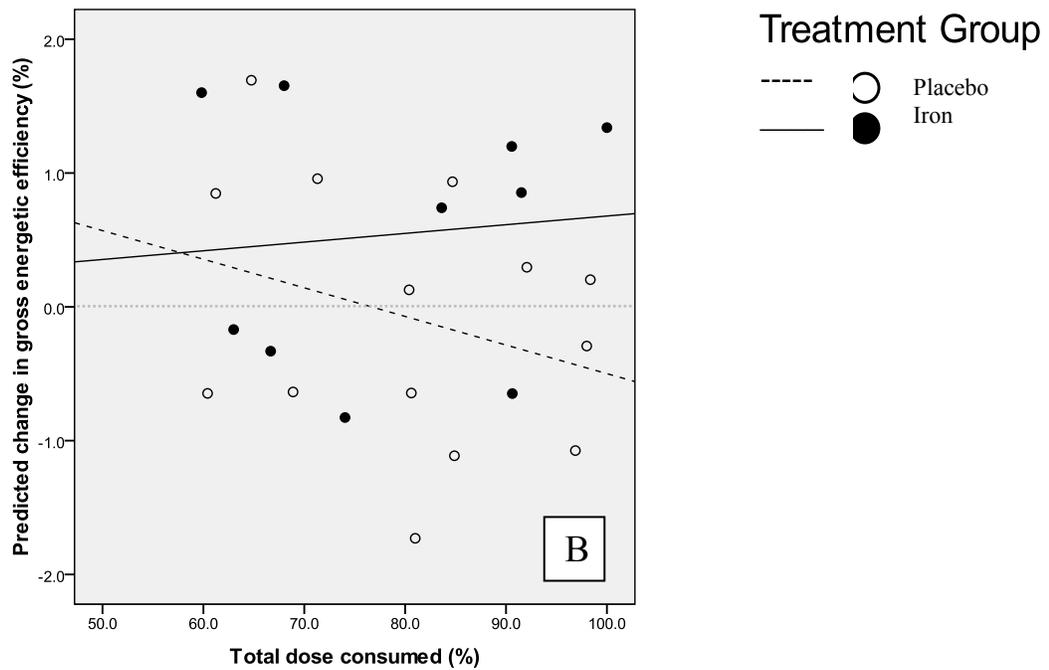
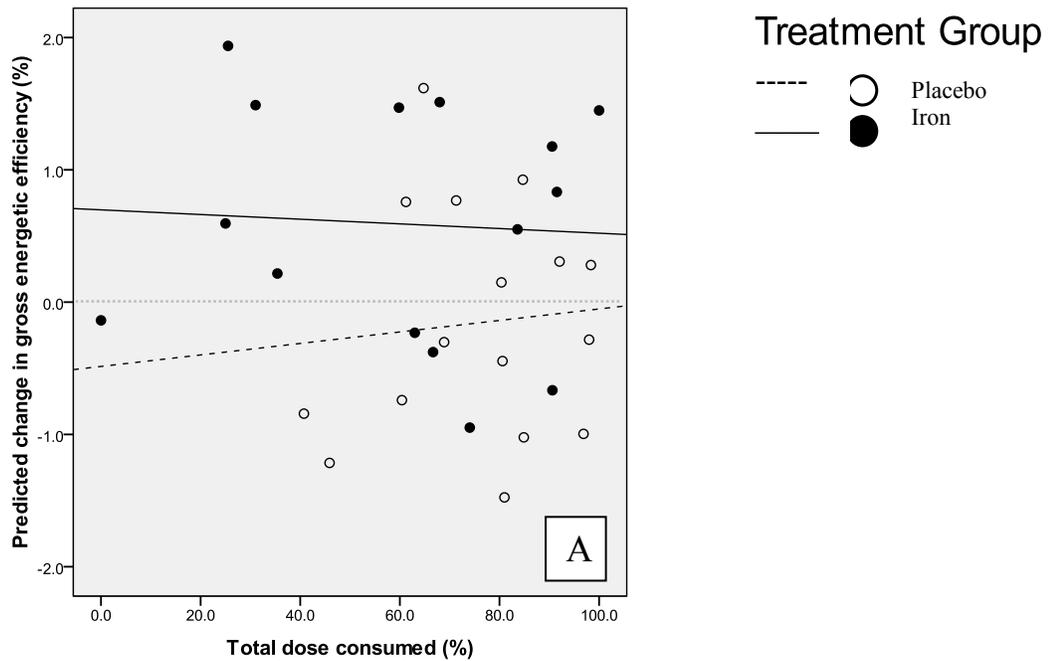


Figure 7.1 A and B. Relationship between dose of supplement consumed and change in gross energetic efficiency in non-anemic rowers. **A**: complete sample ($n=31$, Model 3); **B**: subgroup consuming $>50\%$ of dose ($n=24$, Model 4); both A and B adjusted for treatment group, baseline efficiency, training, dose

To further assess the relative effect of change in iron status on rowers' change in gross energetic efficiency, multiple linear regression analyses were performed with change in gross efficiency as the dependent variable and change in log sFer as the independent variable, controlling for potential confounders such as training, baseline efficiency and supplement dose consumed. Results of the multiple regression analyses testing the effects of change in log sFer are shown in Table 7.5.

Table 7.5. Regression models to test the effects of change in log serum ferritin (sFer) on change in gross efficiency (%) after 6 weeks of training and treatment in entire sample of rowers (n=31)

	Model 4		Model 5		Model 6	
	β	p	β	p	β	p
Constant	3.15	0.15	6.10	0.013	6.05	0.015
Change log sFer from baseline	0.47	0.51	0.44	0.50	0.99	0.48
Dose	-----	-----	-0.02	0.02	-0.02	0.03
Baseline EF	-0.21	0.09	-0.31	0.01	-0.31	0.015
Training group (0=Low, 1=High)	1.44	0.001	1.55	<0.001	1.57	<0.001
Change log sFer*dose	-----	-----	-----	-----	-0.01	0.66
	R ² =0.44 Adj=0.37		R ² =0.54 Adj=0.47		R ² =0.55 Adj=0.45	

In the three regression models, while training and dose of supplement were significant predictors of change in efficiency, there was no independent effect of change in log sFer (Table 7.5, Models 4 and 5 p>0.05). There was no significant interaction between change in log sFer and dose consumed (Table 7.5, Model 6, p=0.66).

Analysis of IDNA subgroup: In order to explain further the plausibility of the relationship between iron status and performance, additional analyses were performed for change in gross efficiency in a subset of rowers (n=16) with baseline sFer <20.0 µg/L (IDNA). Iron status measures for this subgroup are presented in *Chapter 6*, and show that iron supplementation of IDNA rowers improved sFer after controlling for baseline sFer. There were no significant correlations between change in efficiency and any measures of iron status or change in iron status in this subgroup. In the subgroup of those who were IDNA at baseline, those randomized to the iron group were not significantly different from the placebo group in any measure of body composition, training, or performance variable at baseline, with the exception that rowers randomized to the iron group reported significantly less sleep at baseline compared to the placebo group (6.4±0.7 vs 7.3±0.4 hours, p=0.03).

Results of the IDNA subgroup's 4K TT before and after 6-weeks of training and treatment are shown in Table 7.6. After 6 weeks of training, IDNA rowers in both treatment groups improved VO₂peak (+0.2±0.2 l/min, p=0.01) and maximal workload (+20.6±30.9 W, p=0.02), however, there were no significant differences between the two treatment groups except for a change in efficiency in favor of improvement in the iron-supplemented group over the placebo group (p=0.03). There was a significantly positive correlation between change in gross efficiency from baseline to endpoint and total supplement consumed (r=+0.65, p=0.007) in the IDNA subgroup.

Table 7.6. Physical performance measures (4K time, Total VO₂, % VO₂, RER, WR) before and after 6 weeks of training and treatment (n=16 rowers IDNA at baseline)

	Pre-treatment	Post-treatment	Change
4K time, min			
Placebo (n=8)	17.9±1.4	17.7±1.7	-0.2±0.7
Iron (n=8)	17.9±1.3	18.5±2.3	0.6±1.8
Absolute VO ₂ peak, L/min			
Placebo	3.1±0.5	3.3±0.6	0.2±0.2
Iron	3.1±0.3	3.2±0.3	0.1±0.2
Relative VO ₂ peak, ml/kg FFM/min			
Placebo	60.9±4.5	63.2±5.5	2.2±4.3
Iron	64.8±7.2	65.0±5.6	0.2±5.2
Maximal work rate, W			
Placebo	227.8±60.3	253.1±86.9	25.4±35.0
Iron	218.0±21.6	233.9±38.2	15.9±27.7
Maximal heart rate, bpm			
Placebo	187.7±9.6	189.5±9.3	1.8±4.2
Iron	195.2±6.9	194.5±6.9	-0.8±4.4
Maximal RER			
Placebo	1.00±0.04	1.02±0.05	0.02±0.06
Iron	1.03±0.07	1.05±0.07	0.02±0.05
Gross energetic efficiency, %			
Placebo	17.4±2.5	16.9±2.1	-0.5±1.0*
Iron	16.7±1.5	17.5±1.2	0.8±1.1

No significant differences between treatment groups, except for * $p=0.03$ t-test significantly different between treatment groups.

Multiple regression analyses of the rowers with IDNA at baseline are presented in Tables 7.7 and 7.8. Analyses performed with change in gross efficiency as the dependent variable and treatment group as the independent variable showed that after controlling for training, rowers supplemented with iron had 1.1% greater improvement in gross efficiency after 6 weeks of training than did the placebo group (Table 7.7, Model 3, $p=0.02$; w/out training in Model, 1.3% greater than Placebo, $p=0.01$).

Baseline efficiency was not a significant predictor of change in gross efficiency in this subgroup and was left out of subsequent regression models.

Table 7.7. Regression models to test the effects of iron treatment on change in gross efficiency after 6 weeks of training and treatment in subgroup of rowers with Baseline sFer<20.0 (n=16)

	Model 1		Model 2		Model 3	
	β	p	β	p	β	p
Constant	-0.50	0.57	3.03	0.23	-0.72	0.45
Treatment (0=P, 1=I)	1.30	0.01	1.16	0.02	1.08	0.02
Baseline EF	-----	-----	-0.20	0.16	-----	-----
Training group (0=Low, 1=High)	-----	-----	-----	-----	0.87	0.06
%Variance explained by Fixed effects						
Within School and Season (residual) variance	75%		75%		59%	
Between-School and between-Season variance	72%		27%		74%	
% of total Variance between (due to) schools and season	33%		62%		32%	

Further analysis with change in gross efficiency as the dependent variable and change in log serum ferritin as an independent variable showed that after controlling for training, change in log serum ferritin did not predict change in efficiency in this subgroup of rowers when used in place of treatment group (Table 7.8, Model 1, $\beta=1.01$, $p=0.31$). There were, however, significant interactions between change in log serum ferritin and the amount of supplemental iron consumed, and change in log serum ferritin and training group (Table 7.8, Models 5 and 6, respectively).

Table 7.8. Regression models to test the effects of change in log serum ferritin and the amount of supplemental iron consumed on change in gross efficiency after 6 weeks of training and treatment in subgroup of rowers with Baseline sFer<20.0 (n=16)

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
	β	p										
Constant	-0.56	0.47	-0.53	0.48	-0.65	0.43	-1.16	0.04	-1.13	0.05	-0.86	0.334
Change log sFer ($\mu\text{g/L}$)	1.01	0.31	0.90	0.40	0.57	0.53	1.64	0.08	1.51	0.08	0.62	0.45
Dose of supplemental iron (mg)	-----	-----	-----	-----	0.002	0.06	0.004	0.009	0.004	0.001	0.003	0.015
Training group (0=Low, 1=High)	1.27	0.03	1.03	0.17	0.56	0.35	0.45	0.41	-----	-----	0.75	0.20
Change log sFer * dose of supplemental iron	-----	-----	-----	-----	-----	-----	-0.006	0.095	-0.006	0.06	-----	-----
Training * change log sFer	-----	-----	1.39	0.61	-----	-----	-----	-----	-----	-----	-5.15	0.12
%Variance explained by Fixed effects												
Within School and Season (residual) variance	23%		17%		41%		58%		62%		54%	
Between-School and between-Season variance	36%		47%		52%		100%		100%		30%	
% of total Variance between (due to) schools and season	61%		73%		56%		63%		62%		36%	

Rowers who improved their sFer status and consumed more than 600 mg of the supplemental iron during the 6-week study (n=5 in iron treatment group) increased their gross energetic efficiency more than those who consumed less than 600 mg of the supplement (n=11, three of those in Iron treatment group; Table 7.8, Model 5, p=0.06 for the interaction, Figure 7.2). Additionally, rowers who improved their sFer status and trained harder over the course of the 6-week trial increased their gross energetic efficiency more than those who trained less (Table 7.7, Model 6, p=0.12 for the interaction, Figure 7.3).

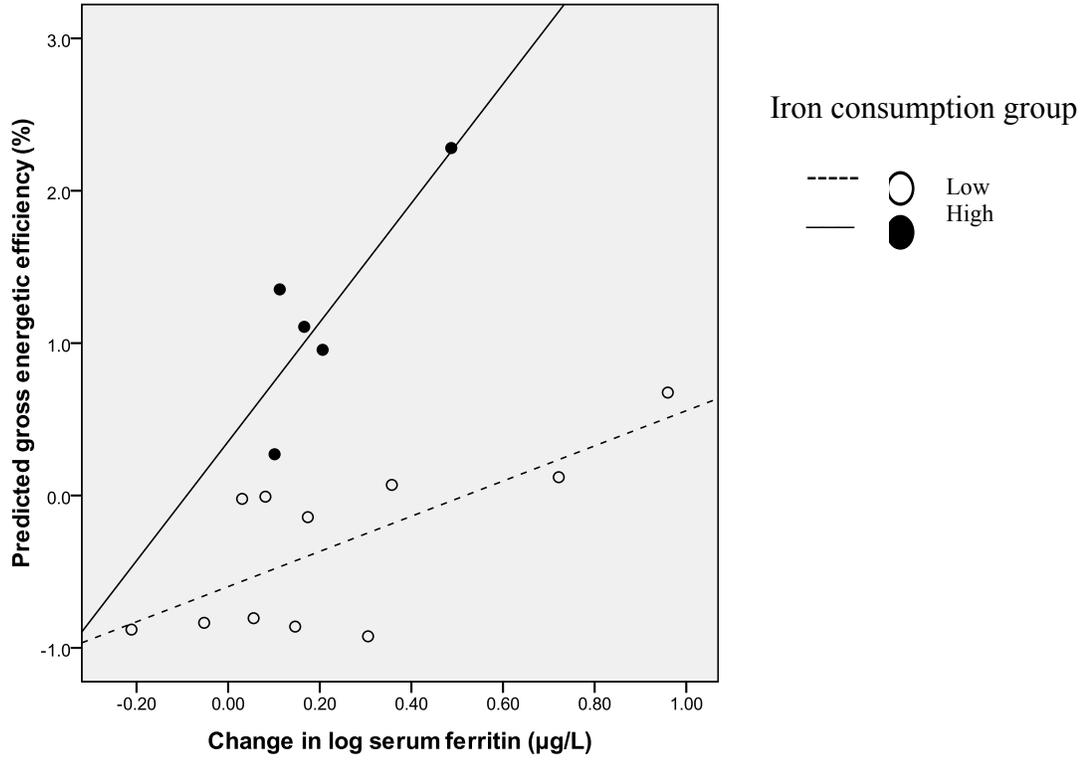


Figure 7.2. Relationship between change in dose of supplement consumed and change in gross energetic efficiency in non-anemic rowers who were depleted at baseline (n=11 in Low consumption group and n=5 in High consumption group). Adjusted for change in log sFer, supplemental iron consumed

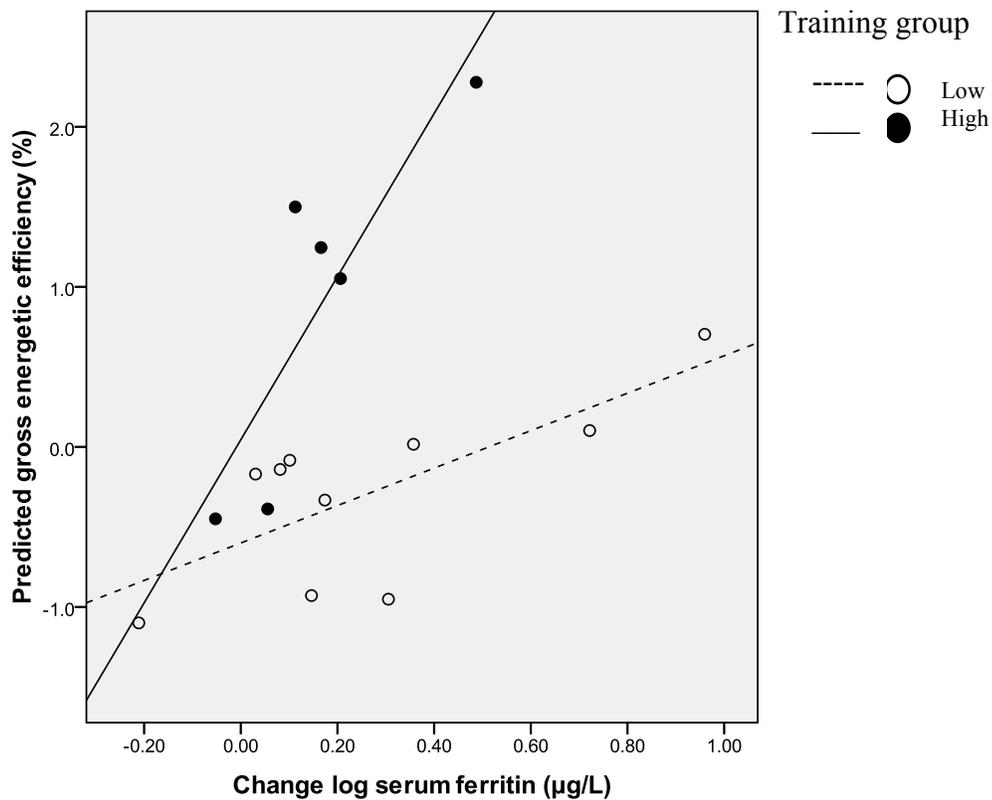


Figure 7.3. Relationship between change in log sFer and change in gross energetic efficiency in non-anemic rowers who were depleted at baseline (n=10 in Low training group and n=6 in High training group). Adjusted for change in log sFer, supplemental iron consumed and training.

Blood lactate: Changes in blood lactate concentration over the course of the 4K TT are shown in Table 7.9. There were no significant differences between the treatment groups in baseline or endpoint pre-test lactate levels. At baseline, after 1000m, lactate increased ~2 - 2.5 fold above pre-test levels during the 4K TT test in the iron and placebo groups, respectively, and remained elevated for the duration of the 4K test. After 6 weeks of training, blood lactate concentrations at all time points during the endpoint 4K TT were significantly lower compared to baseline in both treatment groups (p<0.05).

Table 7.9. Endpoint blood lactate concentration (mmol/dL) during 1K segments of 4K TT before and after 6 weeks of training and treatment

Lactate concentration, mmol/L							
	Pre-test	1000m	2000m	3000m	4000m	5-min post-test	10-min post-test
Baseline							
Placebo	2.3±1.1	8.0±2.2	9.6±2.9	10.4±3.6	13.5±2.0	11.9±2.6	10.0±3.7
Iron	2.8±1.5	7.9±2.6	9.7±3.4	11.2±2.4	13.1±1.2	13.1±1.2	11.2±2.0
Endpoint*							
Placebo	1.9±0.9	5.7±2.0	6.3±2.6	6.7±3.0	11.4±2.1	9.9±2.1	8.3±2.0
Iron	2.2±1.3	5.0±1.8	5.7±2.3	7.0±2.3	12.2±1.7	9.8±3.0	9.0±2.7

* Endpoint lactate values during 4K TT (1000m thru 10-min post-test) significantly different from baseline ($p < 0.05$)

When blood lactate concentration is expressed as a percent of maximal lactate concentration, there was a significant negative correlation between consumption of supplemental iron (total mg) and percent of maximal lactate at the 1000m mark ($r = -0.39$, $p = 0.03$). Further examining lactate expressed as a percent of maximal lactate achieved, there were significant differences between the two treatment groups. Rowers supplemented with iron had a slower rise in blood lactate during the first half of the 4K TT (10% lower than the placebo group), and a faster recovery five minutes after completing the TT compared to the placebo group (8% lower than the placebo group; see Figure 7.4).

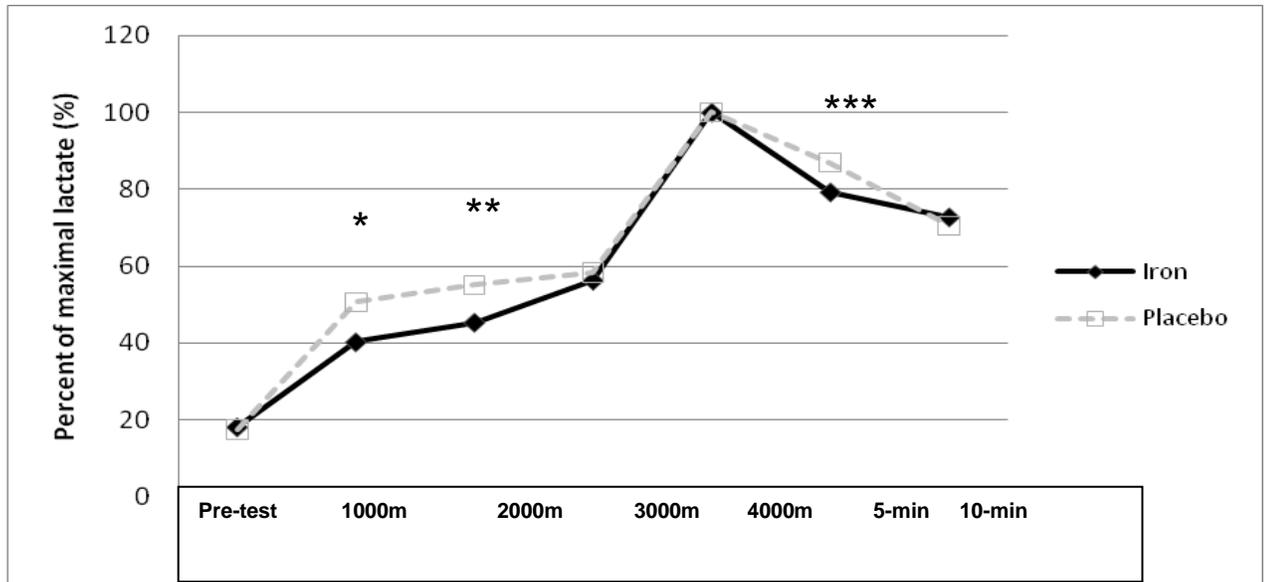


Figure 7.4. Lactate concentration as a percent of maximal lactate at Endpoint (%) between the two treatment groups (n=31; adjusted for treatment group and baseline values). *p=0.001 between Treatment groups (50.6±5.1 in Placebo vs 40.3±9.6% in Iron); **p=0.006 between Treatment groups (55.1±5.4 in Placebo vs 45.4±9.8% in Iron); ***p=0.001 (86.9±5.4 in Placebo vs 79.3±6.4% in Iron).

In the subgroup of rowers with baseline sFer<20.0 µg/L, as a percent of maximal lactate concentration after 6 weeks of training, the Iron-supplemented group had 1000 and 2000 m lactate concentrations that were 12.5% and 13.4% lower, respectively compared to the placebo group, after controlling for values at baseline (1000m: 54.7±9.1 in Placebo vs 42.2±12.8% in Iron, p=0.04; 2000m: 62.3±7.4 in Placebo vs 48.9±9.1% in Iron, p=0.006).

Discussion

The purpose of this experiment was to examine the effects of iron supplementation on endurance performance and training in non-anemic female rowers. Serum ferritin and Hgb were used to identify those to include in the supplementation trial, as done previously (17, 18). Additionally, we measured sTfR and calculated total body iron to differentiate those with low liver iron stores from those with low tissue (functional) iron. After 6 weeks of iron supplementation, rowers were able to improve iron status, after controlling for baseline iron status, and the iron status of rowers with poorest iron status at baseline benefitted the most from supplementation (*Chapter 6*).

In our previous cross-sectional analyses, we found that rowers with IDNA at the beginning of the training season reported slower 2K personal records for the previous season (*Chapter 4*) and had a lower concurrent VO₂peak and reduced energetic efficiency compared to rowers with normal iron status (*Chapter 5*). In the current analyses, time to complete the 4K TT was unaffected by iron supplementation. However, the iron-supplemented rowers were able to significantly increase their energetic efficiency after controlling for baseline efficiency and supplement dose consumed. Furthermore, analysis of data from the subsample of rowers with baseline serum ferritin <20.0 µg/L showed that iron-supplemented rowers clearly increased their energetic efficiency by 1.3% after 6 weeks of training compared to the placebo group.

This means that after being supplemented with iron for 6 weeks, non-anemic rowers were able to perform the same workload at a lower energy cost (lower level of

physical exertion = more energetically efficient). This finding is consistent with similar iron supplementation studies of non-anemic women. Researchers have found that O₂ consumption (as % VO₂max) during an endurance test was significantly less (-3%) after iron supplementation and significantly greater (+3%) in placebo (15). Zhu & Haas showed that after 8 weeks of iron supplementation, non-athletic women increased their efficiency by decreasing their energy expenditure by 5.1% (p=0.016) compared with women in the placebo group, and that this treatment effect on %VO₂peak was mediated by a change in Hgb (17).

Hinton et al found that after a 4-week training program imbedded in a 6-week iron supplementation trial, although both placebo and iron treatment groups increased their work efficiency (through training), there were no significant differences in efficiency between the treatment groups, however, the iron group decreased their O₂ consumption by 5% (as a %VO₂peak) during the last 5K of 15 K TT. (18). Another study of untrained IDNA Mexican women reported 5.2% greater efficiency during a cycle ergometer test after 6 weeks of supplementation with 18 mg of iron (54). Most recently, Hinton et al found that after 6 weeks of iron supplementation, post-trial work efficiency in recreational athletes' was significantly increased (+1.1%) compared to placebo (+0.7%) (20).

The 4K TT test protocol was designed to give rowers an incentive to finish "the race" as they would on the water, similar to the manner in which they train. This familiar format should have minimized the influence of motivational status on test time and level of exertion that is a common problem in time-to-exhaustion protocols. However, there was still a subjective component of the TT protocol, as it was up to

each rower to control her work rate on the ergometer at all times, so despite WR_{Rx} given (85% of max), it was up to each rower to maintain that WR using the monitor on the ergometer. However, deviation from WR_{Rx} was not significantly different between the two treatment groups at baseline or endpoint. Additionally, the difference in energetic efficiency should not have been due to differences in psychological factors between the two treatment groups, as motivation scores measured throughout the trial were not significantly different between the two treatment groups at baseline and endpoint (reported elsewhere, *Chapter 8*).

After six weeks of training, all rowers improved their lactate response during the 4 K TT, however, the iron supplemented group had much slower rise from pre-test lactate (10% lower, as a percentage of maximal lactate) during the first two phases of the TT, and a faster recovery five minutes after completing the test compared to the placebo group. Although many researchers have found no effect of iron supplementation on lactate concentration during exercise in IDNA women (18, 22, 55-57), results from the current study are similar to those of Zhu and Haas who found that IDNA women supplemented with iron showed a slower rise in lactate concentration during the first leg of a 15K TT (5 K mark) on a cycle ergometer, as well as an inverse association between lactate concentration at the 5 K and Hgb, even in marginal iron deficiency (17). Even in the absence of frank anemia (Hgb <12.0 g/dL), impaired O_2 transport capacity due to IDNA appears to affect lactate metabolism, resulting in impaired oxidative metabolism, and ultimately increased reliance on anaerobic metabolism to produce energy (greater lactate production at an earlier stage of

exercise). In a state of IDNA, lactate metabolism may be directly affected, resulting in the prevention or slowing of lactate clearance (31, 32).

Conclusions: Within the limitations of this study, we conclude that IDNA was not found to affect rowers' time to complete a 4K TT on the rowing ergometer. However, after 6 weeks of iron supplementation, rowers' energetic efficiency during the 4K TT was improved compared with the placebo group, after controlling for baseline efficiency and treatment dose. This effect was most evident in the subgroup analyses of rowers with IDNA at baseline. This indicates that IDNA increases rowers' exertion and energy cost to do the same load of work, and that iron supplementation enhanced rowers' adaptation to training. In addition, rowers supplemented with iron showed slower lactate response during the early phase of the 4K TT, and a faster recovery post-TT, indicating that iron depletion affects lactate metabolism, in the absence of frank anemia. Both the energetic efficiency and lactate effects observed are likely manifestations of the same phenomenon related to energy production during exercise.

These results are important for female endurance athletes whose dietary patterns and physical training levels increase their risk of IDNA, and suggest that iron supplementation may maximize the benefits of endurance training. Future studies should focus on implementation of iron status screening programs for female athletes in-training, as well as ways to improve supplement compliance in athletes.

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CHAPTER 8

IRON SUPPLEMENTATION IMPROVES TRAINING QUALITY IN NON-ANEMIC FEMALE ROWERS

Abstract

Introduction: Compared to their sedentary counterparts, female athletes are more susceptible to iron deficiency without anemia (IDNA). Studies in both animals and humans demonstrate a relationship between IDNA and physical performance, however it is unclear how IDNA affects the quality of endurance athletes' physical training, which is integral to athletic performance.

Purpose: To examine the relationship between iron status and training quality, as well as the effects of iron supplementation on the training quality of non-anemic rowers during a competitive season.

Methods: At the beginning of a season, non-anemic collegiate female rowers were randomized to receive either 100 mg/d ferrous sulfate (n=22) or placebo (n=21) for 6 weeks using a double-blind design. Subjects trained with their team as usual and completed daily logs using the Visual Analog Scale (VAS) format to rate aspects of training quality (intensity, concentration, speed, stress). Iron status (hemoglobin, serum ferritin, soluble transferrin receptor, total body iron) was assessed at baseline and at 6 weeks. A training quality score was created for each of the six weeks of training. Change in training quality score (week 6 score minus week 1 score) was used as the dependent variable in mixed multiple regression analyses to examine the impact of iron supplementation on change in training quality.

Results: Twenty-four rowers (n=12 iron, 12 placebo) completed all 6 weeks of training data. After controlling for baseline training quality, multiple regression analysis revealed that rowers in the iron group had an improved training quality score ($\beta=+77.5$, $p=0.03$) compared to those in the placebo group, however, change in iron status (sFer, sTfR, or TBI) did not affect this relationship.

Conclusion: After controlling for baseline training quality, rowers supplemented with iron improved their training quality after 6 weeks of training and treatment compared to those in the placebo group.

Keywords: *Iron depletion, athletes, endurance training, rowers, iron supplementation*

Iron supplementation improves training quality in non-anemic female rowers.

Introduction

Iron deficiency (ID) is the most prevalent nutrient deficiency in the world, including the US, where iron deficiency with anemia (IDA) affects 3-5%, and iron deficiency without anemia (IDNA) affects ~16% of young women (1). Compared to their sedentary counterparts, female athletes are more susceptible to IDNA (25-35%) (2-7). Although the exact mechanism is unknown, the increased prevalence of IDNA in active/training females may be due to a combination of factors, including: hemolysis (foot strike, impact); increased blood loss (gastrointestinal tract, hematuria, sweat); change in iron absorption and/or poor dietary iron intake (8-11).

The iron status of experimental animals and humans is related to their physical training, but the direction of this relationship is unclear (12-18). Animal studies have shown a relationship between ID and physical activity time, frequency, and distance moved (14, 19, 20), and iron's role in the dopaminergic system has been implicated in these activity-related behavior changes. Researchers have also demonstrated decrements in iron status with increased physical training in humans (21-23), though training in these studies had not been quantified or controlled. It has been hypothesized that these declines in iron status with increased training may be related to decreased iron absorption due to inflammation (24, 25), and/or poor dietary intake during training (22, 23). Additionally, we have previously shown that IDNA non-athletic women adapt less to aerobic training (26), and have lower levels of physical activity (27), but evidence in athletes is lacking.

The high prevalence of IDNA among female athletes and the strong association between athletes' iron status and physical performance led us to conduct the current study in which we aimed to provide evidence about a causal relationship between iron status and training in female athletes at the beginning of their competitive season's training program. The objective of this study was to examine the effects of iron supplementation on female collegiate rowers' endurance training. We hypothesized that iron-supplemented rowers would benefit from improved iron status, and that supplementation would prevent or reduce the negative effects of training on iron status, allowing for improved training quality.

Methods

Design: This study was a randomized, double-blind, placebo-controlled iron supplementation trial. Each subject was randomly assigned to a treatment group by a research assistant who was not involved in data collection or contact with subjects. Randomization was done by assigning each subject a random number, with even and odd numbers being assigned to either treatment group. After initial randomization, any imbalance in the distribution of treatment or representation of school or baseline iron status (sFer) was corrected by re-randomization. Rowers were randomly assigned to one of two groups: iron supplementation with 50 mg FeSO₄ twice per day or placebo in the form of identical red capsules. Subjects were provided with 18 capsules each week, and were instructed to consume two capsules per day (four extra capsules were provided). Subjects were instructed to consume one capsule each at their morning and evening meals to minimize potential gastrointestinal side-effects, and with a glass of citrus juice to enhance iron absorption. Subjects were also instructed to

avoid consumption of any other multivitamin/mineral supplements during the 6 week study period.

Compliance with the iron treatment, as well as current health, menstrual status, and physical activity was assessed by daily training logs. Subjects were instructed to record the number of capsules they consumed daily in their log, even if they had consumed less than the prescribed amount per day. Additionally, weekly capsule counts were conducted by the researcher.

Both iron and placebo capsules were prepared by a Registered Pharmacist (PharmD) at the Cornell University College of Veterinary Medicine Pharmacy (Ithaca, NY). The iron supplement capsules contained 50 mg FeSO₄ per capsule with lactose filler, and the placebo capsules contained only lactose. The iron content of both placebo and iron capsules was analyzed via ICP mass spectrometry digestion by the USDA's Robert Holley Center for Agriculture and Health (Ithaca, NY). Twenty capsules were randomly selected for analysis from each of two batches. No differences in the average iron content were found between the two batches of iron-containing capsules (15.8 ± 0.5 mg elemental iron per capsule), and no iron was detected in the placebo capsules.

For all subjects, body composition and physical performance were measured immediately before and after the 6 week treatment period. Thirty-one rowers finished the entire study protocol (all blood analyses and exercise testing) (see Figure 8.1). Six subjects from the iron group and three from the placebo group dropped out of the study due to personal reasons (n=4), injury (n=3) or illness (n=2), all unrelated to the study. Rowers who did not complete the study reported getting more and better

quality sleep at baseline ($p=0.01$ and 0.04 , respectively), as well as feeling better in general ($p=0.03$) than rowers who did complete the study. Compared to the 31 rowers who completed the study, the 9 rowers who did not complete the study had slower times to complete a simulated 4K time trial on a rowing ergometer at baseline (19.4 ± 2.6 min vs 17.7 ± 1.2 min, $p=0.008$). There were no other significant differences in baseline iron status, body composition, training, or performance measures between those who completed the study and those who did not complete the study.

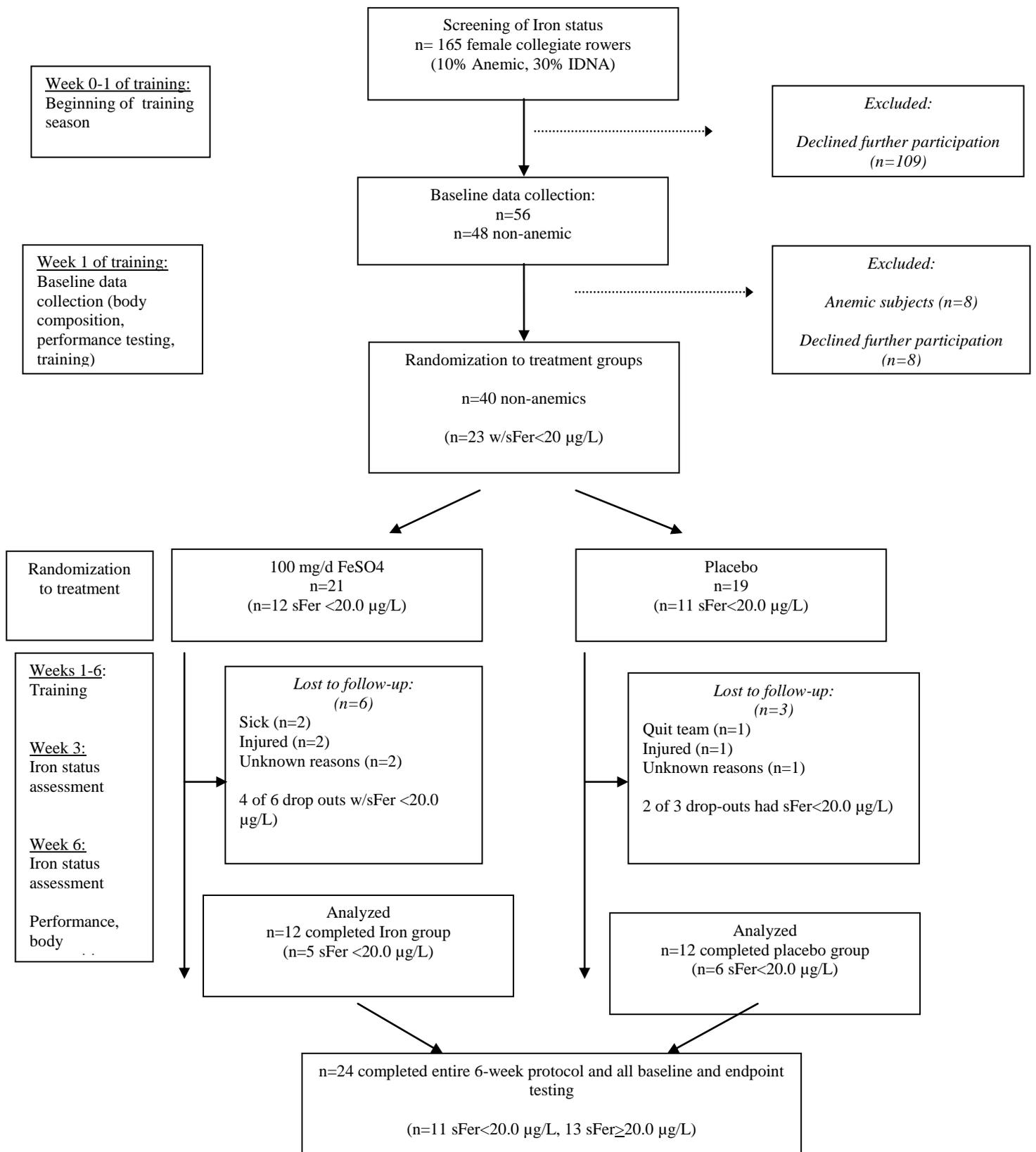


Figure 8.1. Timeline and flow of subjects through randomized, placebo-controlled iron supplementation trial

Assessment of iron status: Iron status variables measured from non-fasting venous blood samples (antecubital venipuncture, into two evacuated tubes, EDTA and serum-separator) included hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC, Beckman Coulter, Fullerton, CA); serum ferritin (sFer, Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL); soluble transferrin receptor (sTfR, Ramco Laboratories, Stafford, TX); alpha-1-acid glycoprotein (AGP, radial immunodiffusion plate, Kent Labs, Bellingham, WA). Total body iron (TBI, mg/kg) was calculated using the ratio of sTfR to sFer as described by Cook et al (28). Every effort was made to obtain baseline, midpoint, and endpoint samples at the same time of day to control for diurnal variation in any measurement of iron status. Hgb and sFer status were analyzed immediately after blood sampling. To control for potential variation in the non-automated sTfR assay conditions, both baseline and endpoint serum samples for the same subject were analyzed at the same time after the supplementation trial was completed. Rowers were classified as either iron depleted (sFer < 20.0 µg/L), normal (sFer ≥ 20.0 µg/L), or anemic (Hgb < 12.0 g/dL). All anemics were notified of their status immediately after blood test results (within one week of analysis), referred to their respective campus health services for further instruction and/or monitoring, and excluded from further participation in the study. All laboratory assays were done in the Human Metabolic Research Unit at Cornell University (Ithaca, NY).

Body Composition: Anthropometric and body composition measurements were determined at the site of exercise testing. Body weight and height were measured with standard procedures and equipment (29). For athletes for whom it was accessible,

body fat and fat-free mass was assessed via air-displacement plethysmography (BodPod, Life Measurement, Inc, Concord, CA). For all subjects, percent body fat was calculated from tricep, suprailiac and thigh skinfold thickness (SF, Lange, Cambridge, MD) (30) and bioelectrical impedance analysis (BIA, RJL Systems, BIA-101) (31). The Siri equation (32) was used to calculate percent fat from body density. For those athletes without access to the BodPod (n=10), an average of their percent body fat values calculated from BIA and SF was used. In a larger sample of subjects with both BodPod and BIA-SF average (n=31), the two methods were highly correlated ($r=0.83$, $p<0.001$), and not significantly different from each other ($p=0.40$). The mean difference between percent body fat calculated from BodPod and the BIA-SF average was $-0.48\pm 3.15\%$ (95% confidence interval of difference: -1.64 , $+0.67$). There were no significant differences in either body fat measurement method across schools.

Assessment of physical performance: Physical fitness and endurance performance was assessed using a rowing ergometer (Concept2, Morrisville, VT) equipped with a digital readout monitor (PM2), displaying work (watts, W), stroke rating (spm), distance (m), and time (min:sec). A computerized metabolic cart (TrueMax 2400, ParvoMedics, Salt Lake City, Utah) was used to measure VO_2 and other physiological measures during all testing. Heart rate (HR, Polar FS2, Polar Electro, Inc, Lake Success, NY) was also continuously monitored throughout testing. Cadence (strokes per minute, spm) and work rate (WR, (W) resistance) were monitored and recorded every 30 seconds. Endurance capacity was assessed at both baseline and endpoint by time to complete a 4K TT, consisting of a 4K ergometer row

at a sub-maximal WR prescription (WR_{Rx}) of 85% of rowers' VO_{2peak} reached in the pre-test. This WR was maintained for 3600m of the test, and the rowers were then asked to sprint the final 400m of the test to simulate on-water racing (Appendix 12). The 4K TT was performed at baseline and endpoint at the same WR Rx. Detailed methods and analysis of laboratory assessments of physical performance (gross energetic efficiency, VO_{2peak} , 4K TT time) have been reported elsewhere (*Chapter 7*).

Assessment of Training Quality: Information on compliance with capsule consumption, current health and menstrual status, usual leisure-time physical activity, and rowing training regimen was quantified each day for 6 weeks via detailed training and activity diaries (Appendix 11). Twenty-four rowers (n=12 iron, 12 placebo) had complete training log data at baseline and after 6 weeks of training and treatment. Questions in the daily log addressed sleep and nap duration and quality, motivation, concentration, soreness and fatigue, and training/physical activity frequency, intensity, time, and type. Eleven questions were presented in the format of a Visual Analog Scale (VAS). Subjects were asked to rate each question by placing a solid vertical line on a 100mm scale anchored by opposing descriptors (see Figure 8.2). All VAS questions were “scored” by measuring the rating with a ruler (mm).

Daily Log Instructions

This training log should be completed on a ***daily basis*** for the next 7 days. Initial use of this log may take up to 5 minutes/day. For some questions, please rate each factor, as you feel ***today*** by placing a ***solid vertical line*** on the scale.

Example: Happy: How happy do you feel right now?

Not at all happy _____ | _____ Extremely happy

Figure 8.2. Visual Analog Scale (VAS) instructions and format of training log

Data Analysis: All data were analyzed using SPSS Statistics version 18.0 (Chicago, IL). Data were examined to verify normality of distribution, and skewed distributions were log-transformed. Results are reported as means \pm SDs. All initial analyses were performed on an “as-treated” basis to examine the effect of iron supplementation on both iron status and training outcomes. Independent Student’s T-test and ANOVA was used to examine treatment group differences at baseline; characteristics differing between treatment groups ($p < 0.05$) were considered potential confounders and were included as covariates in subsequent regression models.

Exploratory principal component analyses (SPSS, Principal Components, Varimax Rotation) was conducted on all training log variables measured weeks one through six to reduce the number of independent variables and improve the strength of the underlying constructs related to quality of endurance training. Data from those variables that loaded together (see Table 8.1, Quality/Intensity Factor, eigenvalue 4.8; 6 items) were then summed, to create a Training Quality score for each of the 6 weeks. The same two factors and similar loadings were observed when analyses were

performed on training data from each individual week. The Soreness/Fatigue factor was not used in any of the present analyses.

Table 8.1. Rotated factor loadings of training variables for all training measures weeks 1 through 6 (average of all ratings from baseline through endpoint)

	Quality/ Intensity Factor	Soreness/Fatigue Factor
Intensity	0.95	-----
Motivation	0.82	-----
Discomfort	0.77	-----
Concentration	0.91	-----
Speed	0.95	-----
Stress	0.93	-----
Soreness	-----	0.84
Fatigue	-----	0.85
Percent of variance explained	87.4	12.6

Repeated-measured ANOVA was then performed on the Training Quality scores to test group and time effects, as well as group-by-time interaction effects for iron status outcomes (baseline, week 3, and endpoint, unless otherwise noted). Repeated measures models of training quality included weeks 1-6 (baseline through endpoint). Pearson’s correlations were used to examine the relation between change in iron status and change in training quality.

Mixed linear regression analysis, including both random and fixed effects, was used to assess the effects of iron supplementation on change in training quality. School was treated as a random effect to control for unmeasured potentially confounding factors related to school differences in iron status. A statistical significance level of $p < 0.05$ was the level of statistical significance for main effects, and $p < 0.20$ for testing interaction effects.

Results

Subject characteristics: The placebo and supplemented groups were of similar age (19.9 ± 1.2 and 19.8 ± 0.8 years, respectively) and height (171.7 ± 7.3 and 170.2 ± 5.3 cm, respectively), and had similar years of rowing experience (3.6 ± 2.4 and 2.4 ± 1.1 years, respectively). Body weight and composition did not differ between the two groups before or after the study (Table 8.2). Rowers in both groups significantly increased their FFM by 1.0 ± 1.2 kg and decreased their percent body fat by 1.1 ± 1.6 % after 6 weeks of training ($p<0.001$).

Table 8.2. Anthropometry and body composition of rowers before and after 6 weeks of training and treatment (those with training quality data at baseline and endpoint)

	Pre-treatment	Post-treatment	Change
Weight (kg)			
Placebo (n=12)	69.7 ± 8.9	69.6 ± 8.6	0.2 ± 1.3
Iron (n=12)	67.1 ± 6.1	67.6 ± 6.5	0.5 ± 1.6
Body fat (%)			
Placebo	25.2 ± 4.1	23.8 ± 3.8	-1.4 ± 1.8
Iron	23.7 ± 3.9	22.9 ± 3.9	-0.8 ± 1.3
Fat-free mass (kg)			
Placebo	51.8 ± 4.7	53.0 ± 5.0	1.2 ± 1.2
Iron	51.2 ± 4.5	52.0 ± 4.9	0.9 ± 1.4

NS differences between Tx groups

Compliance: There were no significant group differences in the number of recorded training days (54 ± 9 and 50 ± 17 d in Placebo and Iron groups, respectively) during the study period. The amount of supplement consumed, however, was 25% greater (not significantly) in the placebo group (Table 8.3). There were no differences between the two groups in treatment-associated symptoms (no adverse events or symptoms related to the supplementation were reported).

Table 8.3. Compliance as measured by training log days recorded, weekly pill count

	Placebo (n=12)	Iron (n=12)
Capsules consumed, n	84±19	72±29
Total FeSO ₄ consumed, mg	0	3629±1462
Percent of total Rx consumed, %	78.6±16.7	68.3±25.5

NS differences between Tx groups

Response to iron treatment and relation of iron status to physical

performance: Results of the blood analyses have been reported elsewhere (*Chapter 6*). Multiple regression analyses revealed improvements in body iron stores (log sFer, total body iron) in the iron treatment group after controlling for baseline iron status, and those with most depleted stores at baseline showed the greatest improvement in iron status. Data described elsewhere revealed a significant interaction in the subgroup of rowers with IDNA at baseline between change in iron status and the amount of supplemental iron consumed on change in gross energetic efficiency (*Chapter 7*).

Training quality: Individual characteristics of training quality at weeks one and six (baseline and endpoint) are presented in Table 8.4. After six weeks of training, there was no significant difference between the placebo and iron-supplemented groups in daily training time (64.7±40.3 and 76.9±58.7 min/d, respectively) or session RPE, although the iron-supplemented group reported higher training intensity on average compared to the placebo group (61.0±16.8 versus 50.1±15.1, p=0.08).

Table 8.4. Training quality characteristics before and after 6 weeks of training and treatment

	Pre-treatment (n=24)	Post-treatment (n=24)	Change
Sleep, hours			
Placebo (n=12)	7.4±1.1	7.3±1.1	-0.1±0.7
Iron (n=12)	6.8±0.9	7.0±1.5	0.2±1.6
Motivation			
Placebo	52.5±17.4	54.0±13.0	1.5±14.0
Iron	57.8±13.8	56.6±20.4	-1.1±26.7
Soreness			
Placebo	44.7±12.8	30.5±19.1	-14.2±18.1
Iron	42.2±17.4	27.4±18.2	-14.8±15.4
Fatigue			
Placebo	45.1±17.2	41.7±12.8	-3.4±19.4
Iron	40.9±13.8	42.6±23.9	1.7±21.5
Nap, minutes			
Placebo	53.0±29.7	76.8±33.5	20.1±30.2
Iron	41.8±20.8	60.8±51.8	15.6±34.2
Intensity			
Placebo	50.8±20.9	46.3±14.4 [#]	-4.6±21.6
Iron	51.8±15.1	59.6±15.5	7.7±16.2
Training time/day, minutes			
Placebo	62.3±27.1	53.8±28.0	-8.5±25.4
Iron	63.6±19.1	78.8±63.6	15.1±65.3
SessionRPE			
Placebo	4138.5±2126.3	3431.5±1769.5	-707.0±1761.7
Iron	4191.4±1814.0	5432.9±4207.2	1241.4±4172.3
Concentration			
Placebo	54.1±21.1	51.2±17.2*	-3.0±19.5
Iron	60.4±18.9	70.9±18.9	+10.5±21.5
Speed			
Placebo	45.2±17.3	40.9±15.3**	-4.4±19.7
Iron	48.1±19.2	59.4±16.4	+11.3±19.6
Stress			
Placebo	42.0±17.5	38.8±16.3***	-3.2±20.4
Iron	44.5±14.0	56.8±20.5	12.3±16.6***
Training Quality Score			
Placebo	283.5±101.1	271.7±81.6	-11.7±101.5
Iron	303.8±80.6	355.5±95.6 [^]	+51.8±105.5 ^{^^}

ANOVA between Treatment groups: [#]p=0.04; *p=0.014, **p=0.009, ***p=0.05;

[^]p=0.03, ^{^^}p=0.15 Group*Time

Rowers supplemented with iron increased their training intensity ($+7.7 \pm 16.2$ versus -4.6 ± 21.6 in the placebo group, $p=0.13$) and minutes of training per day ($+15.1 \pm 65.3$ versus -8.5 ± 25.4 minutes in the placebo group, $p=0.26$) from baseline to endpoint, though not significantly different compared to the placebo group ($p=0.13$ and 0.26 , respectively). There were significant endpoint differences in measures of training between the two groups. After 6 weeks of training, the iron-supplemented group reported higher concentration during training ($p=0.03$), as well as greater speed ($p=0.02$) compared to the placebo group. When attributes of training quality were summed into the training quality score, iron-supplemented rowers increased their training quality score from baseline compared to those in the placebo group ($*p=0.15$ for the group-by-time interaction, Figure 8.3).

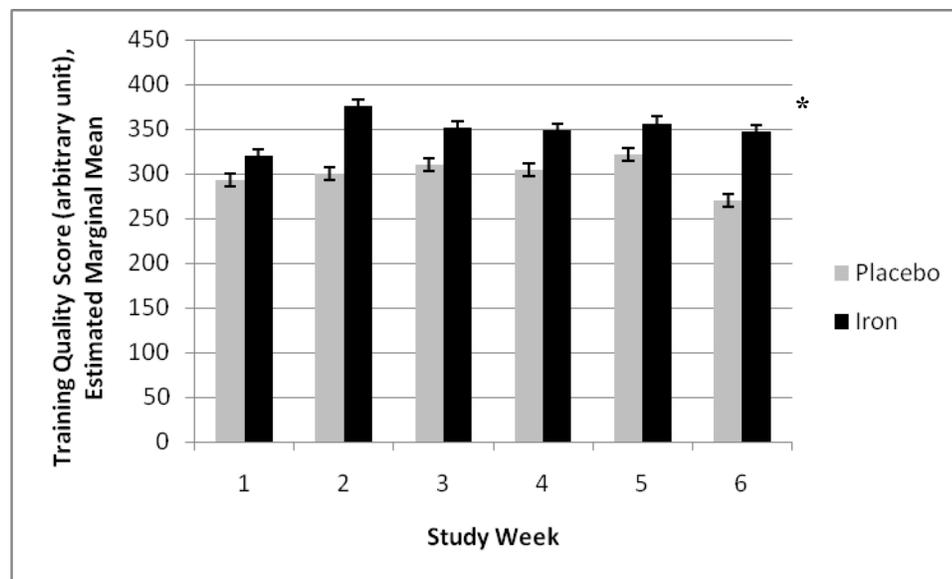


Figure 8.3. Estimated marginal means (EMMs) \pm SE of Training Quality Score (sum of Intensity, motivation, concentration, discomfort, speed, stress) during the 6-week RCT

Iron status and training quality: There was a significant correlation between change in Hgb from midpoint to endpoint and change in total training minutes per day ($r=+0.82$, $p<0.001$). There was a significant negative correlation between change in sTfR and change in fatigue rating from baseline to endpoint ($r=-0.45$, $p=0.03$). There were no significant correlations between change in log Fer or TBI and change in any other training variables examined, nor was there a significant correlation between change in iron status and change in training quality score.

Training and performance: There were no significant correlations between change in training quality score (endpoint minus baseline) and change in gross energetic efficiency ($r=0.16$, $p=0.46$), change in 4K TT time ($r=0.06$, $p=0.79$), or change in VO_2 peak ($r=0.11$, $p=0.60$). Change in motivation and soreness were moderately correlated with change in 4K TT time ($r=0.35$, $p=0.09$ and $r=-0.36$, $p=0.09$, respectively). Change in stress rating was moderately correlated ($r=-0.35$, $p=0.09$), and change in intensity rating was significantly related to change in energy expenditure during the 4K TT ($r=-0.42$, $p=0.04$).

Additional mixed effects regression analyses of the entire sample reveal that after controlling for change in iron status (log sFer) and baseline performance, change in training quality is a moderate predictor of change in gross energetic efficiency ($\beta=0.003$, $p=0.19$, data shown in *Chapter 9*). The interaction between change in iron status and change in training quality was not significant in the regression model. Analyses of the subgroup with IDNA at baseline show that after controlling for change in iron status (log sFer) and baseline performance, change in training quality is a

significant predictor of change in gross energetic efficiency ($\beta=0.009$, $p=0.006$, Figure 8.4).

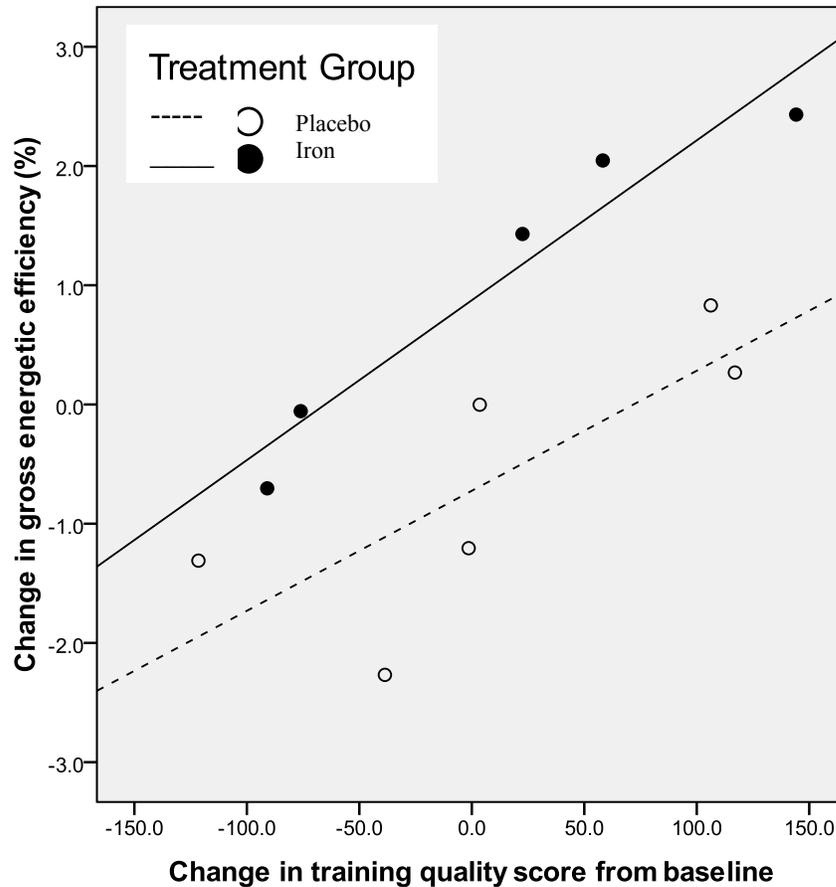


Figure 8.4: Relationship between change in training quality score and change in gross energetic efficiency in IDNA rowers randomized to placebo and iron treatment. Adjusted for change in log sFer and baseline performance.

Change in training quality with iron treatment: To further assess the relative effect of iron supplementation on change in training, multiple linear regression analyses were performed with change in training quality score as the dependent variable and indicators of iron status as independent variables, controlling for potential

confounders such as baseline training and supplement dose consumed. Results of the multiple regression analyses are shown in Table 8.5.

Table 8.5. Regression models to test the effects of iron supplementation on change in training quality/intensity (week 6 minus week1) after 6 weeks of training and treatment

	Model 1		Model 2		Model 3	
	β	P	β	P	β	P
Constant	174.2	0.009	211.05	0.007	171.2	0.04
Treatment (0=P, 1=I)	77.5	0.03	-----	-----	85.6	0.52
Baseline training quality/intensity	-0.67	0.002	-0.65	0.009	-0.66	0.02
Baseline training quality/intensity * Treatment	-----	-----	-----	-----	-0.03	0.95
Change log sFer from baseline	-----	-----	-38.1	0.66	-----	-----
Baseline training quality/intensity * change log sFer	-----	-----	-----	-----	-----	-----
%Variance explained by Fixed effects						
Within School and Season (residual) variance	93.8%		91.9%		93.5%	
Between- School and between-Season variance	55.1%		69.3%		49.9%	
% of total Variance between (due to) schools and season	37.0%		43.0%		34.8%	

Rowers supplemented with iron significantly increased their training intensity from baseline to the end of the study compared to those treated with placebo (Figure 8.5). Dose consumed ($\beta=-0.66$, $p=0.45$), baseline log sFer ($\beta=12.6$, $p=0.85$) and change in iron status, as well as change in either sTfR or TBI, were not significant predictors of change in training, nor were there any significant interactions between iron status and training.

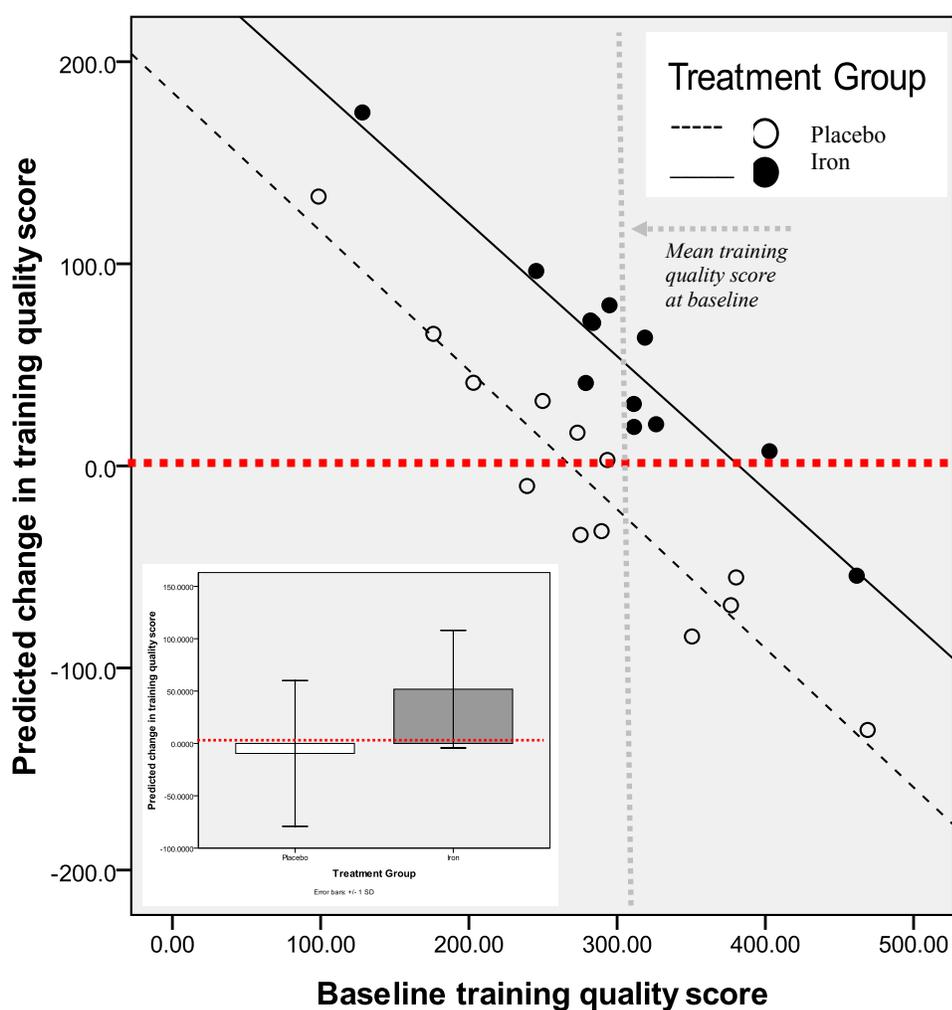


Figure 8.5: Relationship between baseline training quality score and change in training quality score in non-anemic rowers randomized to placebo and iron treatment. Adjusted for treatment group and baseline training quality score (Table 8.5, Model 1).

Discussion

The purpose of this analysis was to examine the effects of iron supplementation on the training quality in non-anemic female rowers. Serum ferritin and Hgb were used to identify those to include in the randomized, double-blind, placebo-controlled iron supplementation trial, as has been done previously (26, 33). Additionally, we measured sTfR and calculated total body iron to differentiate those with low total iron stores from those with low tissue (functional) iron.

In previous cross-sectional analyses, we found that compared to rowers with normal iron status, depleted rowers trained ~10 minutes less per day at the beginning of a training season ($p=0.02$, *Chapter 5*). We have previously reported (*Chapter 6*) that after controlling for baseline iron status, iron supplementation improved iron stores (sFer, TBI). We also found that after controlling for baseline performance, iron supplementation improved endurance performance as measured by gross energetic efficiency (*Chapter 7*).

The relationship between iron status, training, and performance led us to conduct the current analysis, the major finding of which was that six weeks of iron supplementation improved rowers' training quality, as assessed by daily self-report. These findings are important to female athletes because training quality ultimately affects performance. Improvements in all performance and body composition measures indicate that the 6-week training regimens employed by the various rowing teams were successful to induce physiological adaptation. Iron supplementation enhanced this adaptive response to training, as evidenced by greater training quality scores after controlling for baseline training quality.

Training itself has been implicated in changes in the iron stores of endurance athletes (21, 22, 34-36). At the beginning of any type of training program, there is a transient decrease in Hgb and other RBC indices, termed “sports anemia”. This is a temporary condition in which Hgb and RBC indices are diluted due to the rapid increase in plasma volume induced by training (hemodilution) (35, 37-39). As a result, iron is mobilized to tissues to support the demand for RBC production and synthesis of Hgb over iron storage. This condition resolves as the rower adapts to her training regimen during the first few weeks of the training season. In a very small study of elite cross-country skiers (n=2 male and 1 female), training was quantified daily during a 33 week training season (40). Serum ferritin was decreased resulting from either decreased iron stores, or from temporary effects of training adaptation, and MCV increased, reflecting an enhanced number of young RBCs with training. The authors concluded that the large, rapid changes in sFer were likely not a reflection of total body iron stores, and proposed hemolysis and increased basal losses as an explanation, due to the fact that iron stores were not depleted enough to restrict erythropoiesis. However, this may be due to the effect of training on the iron regulatory protein, hepcidin.

Hepcidin has been recently identified as instrumental in iron metabolism (41, 42), and a recent report has reviewed several studies examining the acute increase in hepcidin with intense training (mainly running) (25), and consequent decrease in iron status in active individuals. Female soldiers reported no effect of basic combat training (BCT) on hepcidin activity, although hepcidin concentrations were associated with iron status and inflammation before and after BCT (43). Liu et al found that after

5 weeks of training, rats had lower levels of iron and ferritin, as well as lower expression of DMT1, HCP1, and FPN1 compared to those who had not been trained. They also found that mRNA expressions of hepatic hepcidin and hemojuvelin in skeletal muscle were higher than rats in the control group (44). This animal model shows how the inflammation of training affects iron metabolism via hepcidin's inhibition of iron absorption. Hepcidin was not measured in the current study of female rowers, but remains an important area of future research.

To the best of our knowledge, this is the first report of the effect of iron supplementation on training quality. Studies of military soldiers have reported decrements in iron status with basic combat training, but training outcomes were not reported (23, 45). McClung et al did examine aspects of soldiers' mood and found iron supplementation doubled soldiers' vigor scores (indicator of cognitive status) compared to the placebo group, but there were no differences between treatment groups in any other subscale of the Profile of Mood States (POMS) (23). In a longitudinal study of female runners, Banister and Hamilton showed that iron status varied with fatigue and training load over the course of 300 days (46). Researchers suggest that iron's role in the dopaminergic system may be responsible for effects of iron supplementation on ratings of fatigue, training stress and intensity, as well as those related to cognitive performance, such as motivation and concentration (9, 47).

The training quality score combined several individual aspects of training quality into a single score and appears to perform well as a proxy for training intensity. We did validate the subjective ratings against heart rate during rowing training in a separate sample of rowers (see *Appendix 1*). However, more objective

methods to assess and quantify training, such as measured heart rate and accelerometry, may be more sensitive to changes in iron status and the effects of iron supplementation, and future studies examining the effects of iron on endurance training should employ these methods to quantify training intensity, frequency, and duration during the training season.

At the beginning of the study, there were no differences between schools in training time or intensity ratings, and the training quality score based on PCA did not differ between the five schools. Furthermore, variation in training and performance between schools was accounted for by using a mixed multiple regression model. It is possible, however, that not all of the variance between schools was accounted for using this statistical method. Randomization of rowers to treatment groups was balanced by school, baseline sFer status, and fitness level to minimize (equalize) the effects of group differences between treatment groups. In addition to our small sample size and poor treatment compliance, the success of the training regimen, regardless of iron treatment, led to such significant improvements in fitness that the effect of heavy training may have reduced our ability to detect an effect of iron supplementation or improved iron status on training quality. In previous cross-sectional analyses, we found that iron status differentially affected early-season physical performance according to training level (“low” vs “high,” *Chapter 5*). In the current study, we saw no such interaction between baseline training quality and change in iron status. This may be partly explained by the fact that the margin for improvement with supplementation or improvement in iron status would have been much less in those who trained the hardest (“ceiling effect”).

Conclusions: Within the limitations of the present study, we conclude that six weeks of daily low-dose iron supplementation results in improved training quality in female collegiate rowers during a training season. Female athletes should be screened for iron status at the beginning of a training season. After identification, anemic and IDNA athletes should be provided with supplemental iron, and their iron status should be monitored throughout the training season. Additionally, coaches should take an active role in monitoring training quality over the season, and recommend health status screening as needed.

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CHAPTER 9

GENERAL DISCUSSION

This final chapter has five sections. The first summarizes results from the cross-sectional and randomized controlled trial (RCT) studies. The second examines the internal and external validity of these two studies. The third discusses the biological implications of the major findings from the two studies. The fourth discusses recommendations to athletes, coaches, and collegiate athletic programs. The final section discusses the direction of future research.

Summary of results of Cross-sectional studies and RCT

Results from this study are consistent with previous reports, and add to the evidence that iron status is an important issue facing female endurance athletes. In our cross-sectional study, we have shown that IDNA is prevalent among female collegiate rowers, and that rowers with IDNA reported slower 2K PRs from the previous season. We have also shown that iron status affected rowers' VO_{2peak} differentially, according to training status at the beginning of a season. After adjusting for important covariates, rowers with depleted iron stores had a lower VO_{2peak} , slower 4K time trial (TT) time, and tended to be less efficient during the 4K TT. Performance of rowers who trained less hard at the beginning of a season was more adversely affected by IDNA. There was no effect of poor iron status on performance in the group of rowers who reported training more at the beginning of a season.

In our RCT, we have also shown that rowers who consumed ~15 mg elemental iron per day improved their iron stores (sFer) during training (after controlling for baseline sFer), and that those rowers with the lowest iron stores at baseline benefitted the most from iron supplementation. These findings add to the growing body of evidence that iron supplementation improves the iron status of active women, which may ultimately impact training and physical performance. Furthermore, our RCT has shown that IDNA in female collegiate rowers affects physical performance as measured by gross energetic efficiency. This indicates that compared to rowers with normal iron status, IDNA increases rowers' exertion and energy cost to do the same load of work, and that iron supplementation enhanced rowers' adaptation to training. In addition, rowers supplemented with iron showed lower lactate response during the early phase of the 4K TT, indicating that lactate metabolism is affected in the absence of frank anemia. Rowers supplemented with iron also showed a greater increase in training quality (from baseline to 6 weeks) compared to the placebo group. By the end of 6 weeks, rowers supplemented with iron reported greater increases in training intensity, as well as ratings of concentration and motivation during training compared to the placebo group.

In both studies, lactate response was augmented by normal iron status (cross-sectional study) and by iron supplementation (RCT). Time to complete the 4K TT was unaffected by IDNA status in the cross-sectional study, and by iron supplementation in the RCT. A discrepancy between the two studies was the effect of IDNA on VO_{2peak} , which was found to be lower in IDNA rowers who reported

training less in the cross-sectional study, yet was unaffected by iron supplementation in the RCT.

Critique of validity of the cross-sectional study and RCT

The main objective of these two studies was to investigate how IDNA impairs endurance performance and training in female rowers, and whether iron supplementation can improve iron status, performance and training in this population. To establish this causal relationship between IDNA and performance, three conditions must be met:

1. There must be an association between iron status and performance.
2. There must be a temporal sequence between change in iron status and change in performance.
3. Potential confounders must be controlled.

In this section, we will examine the following to evaluate the validity of the cross-sectional study and supplementation trial in establishing the relationship between iron status and physical performance: internal validity, biological plausibility, and external validity (1).

Internal validity: cross-sectional study: In the cross-sectional study (*Chapters 4 and 5*), we examined the effect of IDNA on performance by comparing performance outcomes (2K PR time, VO₂peak, energetic efficiency, 4K TT time) between IDNA and normal rowers. IDNA rowers reported slower 2K PRs, and those who trained less

at the beginning of a season had lower VO_2 peaks and lower work efficiency compared to IDNA rowers who trained harder.

The cross-sectional study design related performance outcomes to low body iron stores (sFer), but could not allow for establishment of a causal relationship between IDNA and poor performance. A cross-sectional design cannot provide strong evidence about temporal sequence, and not all potential confounders can be excluded. Since this is not an RCT, confounders may not be evenly distributed between the two groups, causing potentially unmeasured bias. Although we tried to control for factors such as height, FFM, and training, we were not able to control for all possible potential confounders between normal and depleted rowers. Any unmeasured confounding factors may mask iron's effects on performance by influencing the sensitivity or reliability of our outcome measurements.

This cross-sectional study enabled us to investigate the plausibility of relationships between iron status, training and performance. This plausibility analysis is useful when there are putative mechanisms to explain how iron status affects physical performance. These analyses suggest relationships, but do not provide causal evidence, as temporal relationships between iron status and performance cannot be determined with the cross-sectional study design. In our heterogenous sample of rowers, we attempted to control for the differences between schools (training regimens, level of competition, rowing experience) using a mixed-effects regression analysis, which included "school" as a random effect, but this only captures some of the unmeasured confounding.

Being related to both iron status and performance, training was examined as a potential confounder, and indeed the IDNA rowers reported training ~10 min less per day, compared to the rowers with normal iron status. To control for the effect of training in the cross-sectional study, training was assessed quantitatively via daily training logs and quantified using the sessionRPE method, and was then controlled for as a covariate in the testing of group differences and in testing for a linear effect of sFer. We examined the rowers in terms of training load at the beginning of a season and divided them into “low” trainers and “high” trainers, based on a sessionRPE cut-off of the 50th percentile (3200).

When designing our study, we hypothesized that training would be a mediator in the relationship between iron status and performance. ID may decrease training, which would then impair performance. In this scenario, controlling for training as a covariate in the analysis of iron status group differences in physical performance would inappropriately over-control for training, and possibly mask the effect of IDNA. In our cross-sectional analysis, training was found to be a modifier of the relationship between iron status and performance, such that the performance of rowers who trained less was more adversely affected by IDNA compared to rowers who trained more.

Because our cross-sectional study was conducted at the beginning of a training season, our “training” measure may be a proxy for another variable related to, but not a substitute for training itself. Examples include motivation, experience, and time and/or accessibility to train off-season. It is likely that those rowers reporting high training at the beginning of the training season are more likely to adapt to the training regimen in spite of their iron status, while those reporting less training still have

potential to increase their training and therefore, their performance. As expected, rowers in the high training group had higher VO_{2peak}/kg FFM ($p=0.01$), and tended to have a faster 4K TT as tested in the lab ($p=0.07$). Unmeasured behavioral and/or psychosocial characteristics of the rowers may explain this scenario, as prior training and rowing experience are important predictors of performance. It could be that rowers with more experience are more likely to train harder despite their iron status, however, there was no difference in rowing experience between the two training groups. If iron status affects the relationship between training and performance, it is only one of the many factors (physiological, psychological, environmental) to be considered.

As a measure of training load at the beginning of the season, sessionRPE (training session intensity rating x training session time) may be a proxy for who came into the season at a higher fitness level due to training on her own prior to the start of the season. This could be related to self-motivation or any of the other factors mentioned previously. Self-motivation is also an important variable, as it drives the training stimulus, and consequently performance. In a study of female college rowers, those who did not comply with the prescribed training regimens (e.g. comparable to our low training group) had lower self-motivation and poorer ergometer performance than those rowers who trained harder (e.g. comparable to our high training group) (2). In the current study, motivation scores were not different between the depleted and normal rowers, but more highly-trained rowers reported significantly higher motivation scores (58.5 ± 11.9) compared to less highly-trained rowers (49.6 ± 15.9 , $p=0.03$). Rowers in the high training group also reported higher ratings of

concentration during training (63.5 ± 11.9) at the beginning of a training season compared to the low training group (52.3 ± 14.2 , $p=0.005$). SessionRPE at the beginning of a training season may also be a proxy for who performed well during the previous season. Rowers' in the high training group reported 2K PRs from the previous season that were, on average, 17.2 seconds faster compared to the low training group ($p=0.06$).

Internal validity: Given the limitations of the cross-sectional analysis, it was important to conduct a randomized, double-blinded, and placebo-controlled trial (RCT) to further examine the relationship between iron status and supplementation and performance in these athletes. The RCT design lends to better internal validity than the cross-sectional study in establishing causal relationships, as the RCT design meets all three of the previously mentioned criteria. Randomization was successful in that all potential confounders were theoretically evenly distributed between treatment groups, and there were no baseline differences in any performance outcome, iron status, or confounding variables. Successful randomization could be an explanation for a lack of a training effect (as a mediator or modifier) in the RCT. Our training variable was equally distributed between the two treatment groups at baseline.

External validity: External validity is especially relevant for negative or non-significant findings, which may be due to effect modifiers inherent to the study population. These factors may have directly interfered with the effectiveness of the iron supplementation, making the treatment insufficient to cause changes in iron status, performance or training outcome measurements. The generalizability of these findings to other populations of women with IDNA is limited due to our inclusion

criteria, which limited participation to female collegiate rowers who agreed to participate in the study. The results of the supplementation trial would not be appropriately applied to populations that have higher iron requirements due to excessive iron loss and/or poor iron absorption (unhealthy, clinical populations). The duration of our RCT and dose of supplemental iron may not produce the same results in a clinical population.

These results are consistent with results observed in other similar populations, and are likely applicable to other well-trained female endurance athletes (runners, swimmers, skiers, etc), due to the commonality of the college/university environments, and general nature and common outcomes of their training. It is likely that well-trained female athletes across sports disciplines are more prone to IDNA for similar reasons (poor dietary intake, high training load), and that their mitochondrial respiratory potential (iron-containing enzymes involved in oxidative metabolism) would also be compromised. Furthermore, well-trained athletes of all sports disciplines can be highly motivated during exercise testing, so the effect of large variation in motivation or other similar psycho-social characteristics would be minimized. The results from these studies may not be applicable to non-athletes, as underlying genetic, psychological or sociological characteristics between athletes and non-athletes may differ (eg. motivation, etc).

Does training act as a mediator of the effect of iron status or supplementation on performance? To answer our main research question (refer to *Chapter 2*, Figure 2.4, Conceptual diagram) four conditions must be met (3) : 1) *Iron status* is significantly associated with *performance* (establishes evidence that there is an effect that could be mediated by *training*); 2) *Iron status* is significantly associated with *training* (treating training as an outcome variable); 3) *Training* is significantly associated with *performance* (after controlling for iron status); 4) The significance of the impact of *iron status* on *performance* is reduced after controlling for *training*. These four conditions will now be individually-discussed, referring back to tables and figures from *Chapters 6, 7, and 8*.

1) *Iron status* is significantly associated with *performance* : Multiple regression analyses of the subgroup of rowers with IDNA at baseline revealed a significant interaction between change in iron status (log sFer) and the amount of supplemental iron consumed, and the change in iron status and training load (see *Chapter 7*, Table 7.7, Models 5 and 6; Figures 7.4 and 7.5). These effects were not seen in the whole sample of rowers, likely due to the inclusion of rowers with normal iron status. Results of the subgroup analysis provide evidence that there is a relationship between iron status and performance that may be mediated by training.

2) *Iron status* is significantly associated with *training*: Change in training quality as the outcome was not significantly correlated with change in iron status, and multiple regression analyses revealed that change in iron status (as measured by log sFer, sTfR, or TBI) was not a significant predictor of change in training quality score

(see *Chapter 8*, Table 8.5, Model 2). This remained true in the subgroup of rowers who were IDNA at baseline.

3) *Training* is significantly associated with *performance*: Multiple regression analyses of the entire sample of rowers (n=31) revealed significant effects of rowers' average training load, baseline energetic efficiency, and dose of supplement consumed on change in performance (energetic efficiency), but no significant effect of change in iron status (log sFer) on change in performance. Furthermore, there was no interaction between change in iron status and average training load (see *Chapter 7*, Table 7.5). Additional mixed effects regression analyses of the entire sample reveal that after controlling for change in iron status (log sFer) and baseline performance, change in training quality is a moderate predictor of change in gross energetic efficiency ($\beta=0.003$, $p=0.19$). The interaction between change in iron status and change in training quality was not significant in the regression model. Analyses of the subgroup with IDNA at baseline show that after controlling for change in iron status (log sFer) and baseline performance, change in training quality is a significant predictor of change in gross energetic efficiency ($\beta=0.009$, $p=0.006$). Results of the subgroup analysis provide evidence that there is a relationship between training and performance. The relationship between change in training quality and change in performance (gross EF) between treatment groups in the entire sample (A) and the IDNA subgroup (B) is presented in Figure 9.1.

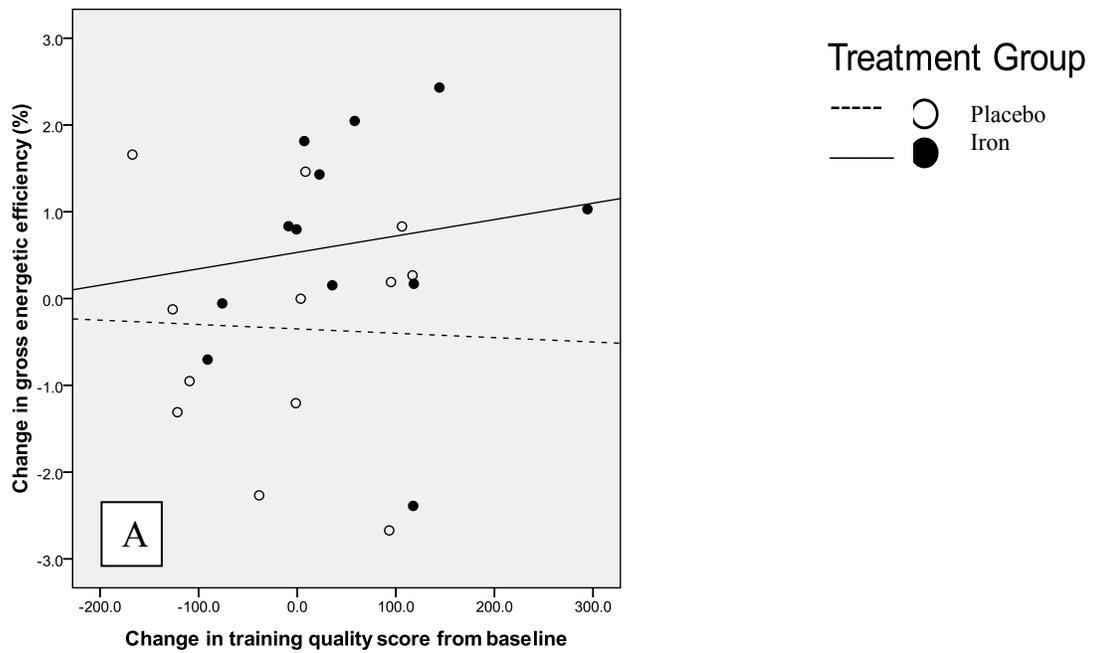


Figure 9.1A. Relationship between change in training quality score and change in gross energetic efficiency between treatment groups in the complete sample ($n=31$).

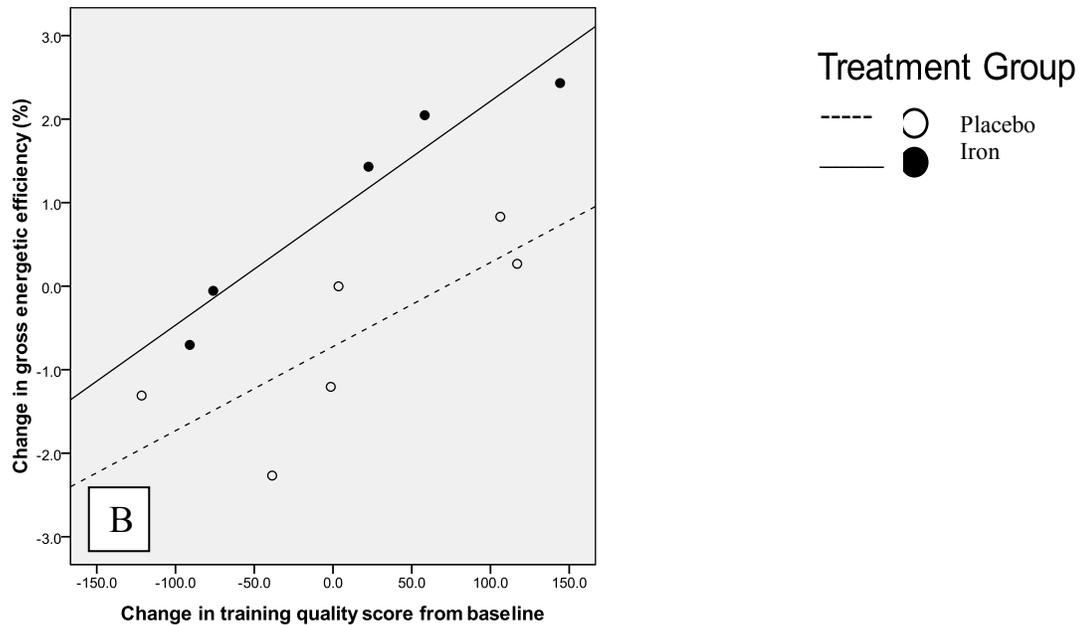


Figure 9.1B. Relationship between change in training quality score and change in gross energetic efficiency between treatment groups in the subgroup of rowers with IDNA at baseline ($n=16$); both A and B adjusted for change in iron status, baseline efficiency

4) The significance of the impact of *iron status* on *performance* is reduced after controlling for *training*: Further analyses suggest that the effect of change in iron stores or iron supplementation on change in gross energetic efficiency may be partially-mediated by change in training quality. This is evidenced by a decrease in the effect of change in log sFer ($\beta = -0.53$, $p = 0.64$ without training, versus $\beta = -0.82$, $p = 0.46$ with training), as well as a decrease in the effect of treatment group (without change in iron stores in model, $\beta = 0.64$, $p = 0.15$ without training, versus $\beta = 0.37$, $p = 0.54$ with training) after adding change in training quality to the mixed effects multiple regression models controlled for baseline training quality. Although these effects were not statistically significant, it is important to further examine the simple relationships between the independent variable (change in sFer), mediator (change in training quality), and the dependent variable (change in gross energetic efficiency), which are presented in Figure 9.2.

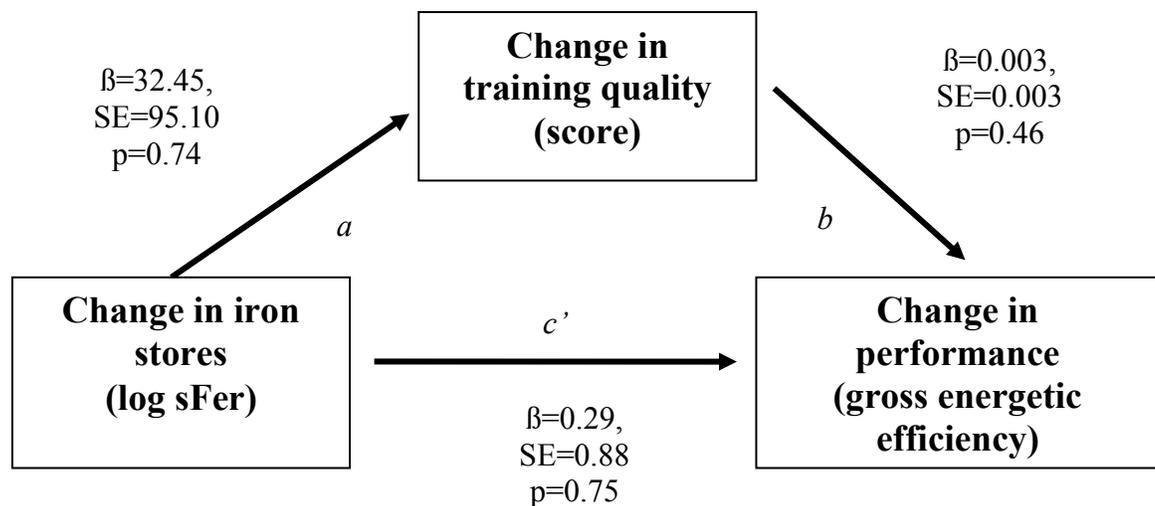


Figure 9.2. Regression analysis to test for mediation

The Sobel method (3, 4) is one way to test whether change in training quality carries any of the influence of the effect of change in sFer on change in gross EF. Although the un-standardized β -coefficients are not different from zero, results of the analysis reveal that ~ 25% of the direct effect (change sFer \rightarrow change gross EF) is mediated by change in training quality (Sobel test = 2.65, $p=0.008$, ratio of indirect/direct effect=0.32). However, the Sobel method is very conservative, and our small sample size may affect the validity of (add bias to) this estimate.

Especially in a small sample, multi-colinearity, or strong correlation between change in iron stores (independent variable) and change in training quality (proposed mediator) may bias regression coefficients and thus interpretation of the mediation effect. However, the correlation between change in iron stores (log sFer) and change in training quality was not significant ($r=0.07$, $p=0.74$), so this was not a major concern in this analysis. Furthermore, if our measure of training quality (proposed mediator) is not completely free of error, estimates of the regression coefficients will be biased (effects of b and c'). Analysis of our training quality score over the 6-week study showed high internal consistency (Cronbach's alpha = 0.82, $n=21$ with complete data for training weeks 1 through 6).

After controlling for baseline training, change in gross EF was a significant predictor of change in training quality ($\beta=26.74$, $p=0.047$). When change in iron stores (log sFer) is added to this model, there is no change in gross EF's effect on change in training; when treatment group is added to the model, the effect of change in gross EF on training is decreased ($\beta=19.02$, $p=0.15$), and treatment has a modest effect ($\beta=60.31$, $p=0.09$). Theoretically, this reverse causality (change EF \rightarrow change

in training) does not make sense -- those rowers who improved their EF, should have done so only after training (temporality). There may be a different variable that is known to cause each of these two variables independently (a variable that causes change in training, but not change in EF, and vice-versa). Examination of instrumental variables would help us to better estimate mediation. Alternatively, omission of any variables that cause both change in EF and change in training will bias the estimates, but since this was a RCT, this should not be the case.

Although the RCT is a strong study design, it is not immune to limitations, introducing insult to the study's validity. It is possible that the physical performance and/or training measures used in this study may not have been sensitive enough to demonstrate any improvements in highly-trained IDNA athletes. A ceiling effect at this moderate (non-clinical) stage of ID on performance is possible. Additionally, an overwhelming response to heavy training may have masked effect the effect of iron treatment or change in iron status. Other possible limitations include selection bias (recruitment and attrition of study subjects), measurement bias, confounding bias, and power, which are discussed below.

Selection bias and recruitment and attrition of study subjects: We expected a 5-10% refusal rate of potential subjects during subject recruiting, and a 10% drop-out rate during the 6 week RCT. Out of 199 possible rowers from the 5 schools, 83% participated in the screening (13% refusal rate, *Chapter 4*). From the 165 that were screened, only 34% (n=56, across range of iron status) opted to participate in the cross-sectional testing (*Chapter 5*), and 24% (n=40 non-anemics) were randomized into the 6-week study (*Chapters 6-8*). A possible explanation for the low response

rate and participation in the baseline laboratory testing and RCT may be the initial time required at baseline. Although obtaining the blood sample for analysis of iron status took less than 5 minutes, completion of the screening questionnaire packets took about 30 min, and may have been perceived as burdensome. Also, commitment to participation in the baseline laboratory testing required 2 separate visits to the lab (VO₂peak and 4KTT tests). Although our subjects received all iron status, body composition, and fitness test results, we did not financially-compensate our subjects, and this may have deterred participation. At the end of the study, subjects who completed the trial were debriefed and asked if monetary compensation would have increased participation; 84% of these participants answered “No.”

For the RCT, the attrition rate was 23%, but the 9 rowers who did not complete the trial were no different from those who did complete the trial regarding their baseline characteristics (for variables we could examine). Bias may occur when those who refuse to participate in the study or drop out during the study are different from those that joined and remained in the 6 week study, relevant to risk factors and behaviors associated with the outcomes. We attempted to minimize attrition by frequent contact with research subjects via bi-weekly email reminders of testing appointments, supplement and training log completion, and weekly face-to-face contact to collect/distribute capsules and logs, as well as to remind subjects of the availability of investigator(s) to answer questions prior to and during the trial, and regular, personalized interaction between research staff and subjects.

Additionally, we attempted to maximize clarity of subject instruction with regards to training logs and supplement intake. Furthermore, accommodations were

made for transportation to/from and parking at the HMRU, as necessary for blood samples, exercise testing and body composition assessments. Selection bias was also partially controlled through successful randomization to treatment and placebo groups, and the double-blinded design protected our study against treatment-associated biases, helping us to avoid subjectivity in outcome measurements, treating subjects differently based on treatment, etc. However, even after randomization, there is still the possibility that unmeasured confounders were not uniformly distributed between groups.

Measurement bias: Recall bias is inherent in the FFQ and other self-reported dietary and behavioral assessment methodology. To help mitigate the effects of this bias, detailed instructions and formats were provided for the diet and training logs. Researcher bias during testing and anthropometric measurements was minimized by blinding the data collector to the participant's treatment assignment and using structured interview guides as necessary.

Confounding bias: There was a risk of confounding due to the interrelated nature of the multiple determinants of iron status in female collegiate athletes. Some of this potential confounding was controlled through the exclusionary criteria (women younger than 18 and older than 30 y; women using substances known to affect outcome variables (smoking, alcohol, etc). We attempted to measure potential confounders related to dietary intake, supplement intake, and intervention compliance, and adjusted for these as appropriate during statistical analysis. Our randomization was successful in regards to measured confounders, and should have accounted for unmeasured confounders.

We attempted to adjust for any *unmeasured* potential confounders using mixed effects multiple regression analyses, which takes into account the heterogeneity between subjects. In a mixed model, intra-individual (random variation within an individual) and inter-individual (random variation among individuals) variability are characterized. We used school and season as our random effects, as it was inevitable that there be inherent differences in factors affecting training and performance at the school and season levels, however, this only captures some of the unmeasured confounding.

Power and statistical analysis: Though we expected similar effects of IDNA on endurance as we have observed in our previous studies (5-8), the current study was underpowered to detect differences less than 1 SD of change in endurance performance, due to our small sample size of IDNA rowers. Additionally, athletes would be expected to have a smaller margin for improvement in endurance training and performance due to their continuous high levels of training. Furthermore, compliance (amount of supplement consumed) was low, and we had fewer IDNA rowers complete the trial than we had anticipated. Due to the exploratory nature of many of our analyses, numerous statistical tests were performed, increasing our susceptibility to Type I error.

Plausibility

In both the cross-sectional study and the RCT, significant associations between change in physical performance and iron status or change in iron status were observed, making it plausible that iron status affects physical performance in female rowers. In the cross-sectional study, there were significant group differences (IDNA vs normal) in VO₂peak, which was strongest in those rowers who reported the least intense training at the beginning of a season. This difference was seen using a sFer cut-off of <20.0 µg/L. Furthermore, there was a significant positive association between VO₂peak and sFer (r=0.29, p=0.05), making it not only plausible that the group difference in VO₂peak was due to group differences in sFer, but it also strongly supports the inference that VO₂peak may be impaired in IDNA, despite non-clinical diagnosis.

If there were a significant correlation between change in iron stores (TBI) and number of iron capsules consumed in the iron supplemented group, this would confirm that the observed change in iron status was due to the iron supplementation, further increasing the plausibility of the results. Rowers in the iron group, on average, consumed 76% of the total prescribed dose of iron (4200 mg FeSO₄), which may not have been enough to see significant differences in iron stores (sFer) over 6 weeks of supplementation. sTfR did decrease in the iron-supplemented group, though not significantly so. Consequently, there was an insignificant change in TBI in both groups (Placebo group +0.45±2.5 mg/kg; Iron group +1.3±3.0 mg/kg, p=0.37), though it appears to be in the expected direction.

The relationship between supplemental iron consumed (mg elemental iron per kg of body weight) and change in total body iron (mg/kg) was discussed in *Chapter 6*, and is shown in Figure 9.3 A and B. Figure A shows the entire sample of rowers (n=14 iron, 16 placebo), less one outlier who was a non-compliant rower in the iron treatment group with a large increase in TBI. After controlling for TBI at baseline ($\beta=-0.58$, $p<0.001$), total supplemental iron consumed (0 mg in the placebo group) was a moderately significant predictor of change in TBI ($\beta=0.07$, $p=0.13$; $R^2=0.56$). Examining those rowers in the iron group separately (n=14), total supplemental iron consumed remained a moderately significant predictor of change in TBI ($\beta=0.12$, $p=0.18$; $R^2=0.51$). Figure B shows the subgroup of rowers (n=7 iron, 8 placebo), less the same outlier who was a non-compliant rower in the iron treatment group with a large increase in TBI. In the subgroup of rowers who were IDNA at baseline, after controlling for TBI at baseline ($\beta=-0.68$, $p=0.002$), total supplemental iron consumed was a moderately significant predictor of change in TBI ($\beta=0.11$, $p=0.10$; $R^2=0.59$). Examining those IDNA rowers in the iron group separately (n=6), total supplemental iron consumed became a significant predictor of change in TBI ($\beta=0.23$, $p=0.05$; $R^2=0.79$).

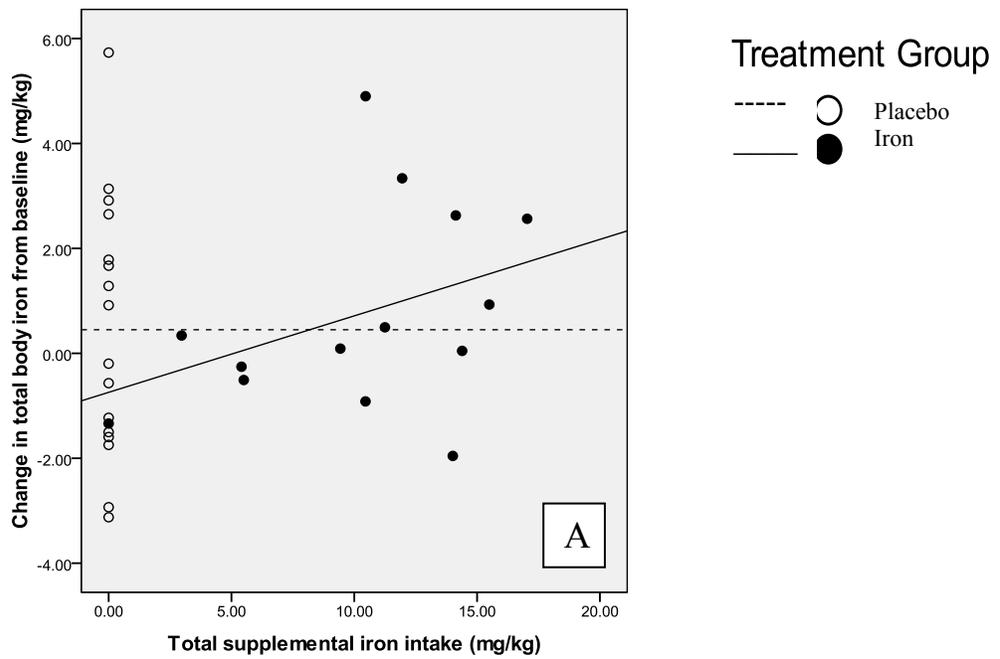


Figure 9.3A. Relationship between supplemental iron intake (mg/kg) to change in total body iron (mg/kg) in A) all iron-supplemented rowers (n=30)

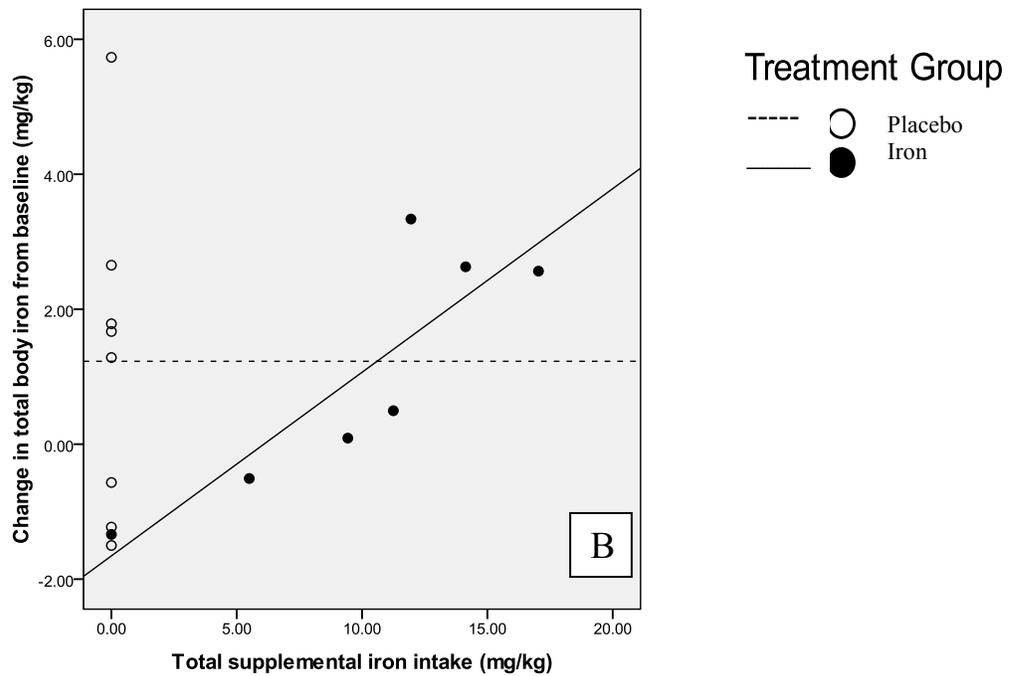


Figure 9.3B. Relationship between supplemental iron intake (mg/kg) to change in total body iron (mg/kg) in iron-supplemented rowers with IDNA at baseline (n=15)

More infrequent (weekly) supplementation with 60 mg of iron for 7 months has been shown to be as effective as daily supplementation in increasing sFer in IDNA premenopausal women (9), and several researchers report improved iron status and better compliance with this mode of supplementation in pregnant women and children (10). In the current study, compliance did not reach >50% until the midpoint (week 3, 11 ± 4 capsules per week in the placebo and 10 ± 5 in the iron group) of the trial, before which rowers were only consuming ~20-40% of the prescribed iron dose. This non-compliance persisted despite several efforts by the research team to demand compliance (weekly reminder emails and weekly visits to collect unused capsules and training logs). Potential explanations may be that it took these women longer to make taking the capsules part of their daily routines due to the demands and schedules of student athletes, or the fact that not many of the subjects were used to routinely taking supplements or medications of any kind prior to the study. At baseline, there were no differences between treatment groups in prevalence of past supplementation. Furthermore, compliance did not differ according to baseline iron status.

Compliance with the supplement was poor, although no adverse effects related to the capsule consumption were reported at any time during the study. On average, rowers in the placebo group were more compliant (79 ± 19 capsules, range 44-115 capsules), and consumed a greater number of capsules than rowers in the iron group (64 ± 34 capsules, range 0-118 capsules). At week 6, rowers in the placebo group were consuming significantly more capsules per week (12 ± 2) compared to the iron group (8 ± 5 , $p=0.04$). If we had given a larger dose of iron, or supplemented for a longer period of time to compensate for the less frequent consumption, we may have seen a

greater improvement in iron stores. Alternatively, at the same consumption rate we may have seen greater improvement in iron stores after 9 weeks of supplementation compared to 6 weeks.

Another test of plausibility is an examination of subjects' potential to benefit from the treatment. In the intervention study, rowers with the poorest iron stores (sFer, TBI) had the greatest improvement in iron stores with supplementation (*Chapter 6*). This is a finding we expected given the regulation of iron absorption according to iron status. However, there was no correlation between supplemental iron consumed and change in body iron stores (log sFer, TBI) in either the entire sample of rowers, or the subgroup with IDNA at baseline. This was likely due to the combination of poor compliance (did not consume enough iron) and small sample size of IDNA rowers who were most likely to respond to treatment.

We observed significant treatment effects on change in gross efficiency and in endpoint lactate response during the 4K TT. Although we observed no correlation between change in efficiency and total supplemental iron consumed, we did find a significant correlation between supplemental iron consumed and lactate (as a percent of maximal) at 1000 m at the end of the study ($r=-0.39$, $p=0.03$), confirming plausibility that the improvement in lactate response in the iron group was due to the supplementation.

Discussion and interpretation of major findings

Iron status

The iron-supplemented group was able to replenish their iron stores (sFer, after controlling for baseline sFer), and those with the lowest sFer at baseline benefitted most from supplementation. However, there were some subjects in the placebo group who had improved iron status. Some of these “responders” in the placebo group could be a statistical artifact of regression to the mean. It is possible, however, that those placebo “responders” truly improved their iron status by other means, such as increased dietary intake during the training season. At baseline, there were no differences in energy intake (2000 kcal/d) or iron intake (18 mg /d) between the two treatment groups. We did not collect weekly dietary logs throughout the 6- week study, and very few rowers returned a completed 7-d food record at the end of the study. With an increase in training load over the course of the season, it is reasonable to believe that kcal intake would have increased, along with dietary iron intake, so it is possible that “responders” in the placebo group may have had higher dietary iron intakes that resulted in improved iron status.

Our RCT included randomization of rowers with normal iron status into the treatment/placebo groups. Another RCT of female soldiers (11) reported improved iron stores (or a prevention of decline in iron stores) with the same supplementation level used in the present study (100 mg/d FeSO₄) in subjects with sFer as high as 37 µg/L. In the current study, although rowers with IDNA and normal iron status were balanced between the two treatment groups, a consequence of including potential “non-responders” reduces the ability to see a treatment effect (change in iron stores) in

the iron group. Duplicate measurements of iron status were conducted at baseline under standard laboratory procedures, along with an indicator of inflammation (AGP), so chance of misclassification of IDNA subjects was reduced, however, initial misclassification of IDNA rowers may still have existed due to use of cut-off values for iron status measurements that are continuous, measured with error and show day to day variation, and don't consider individual sources of normal variation. Furthermore, all rowers in this study had Hgb above the clinical cut-off, and 6 rowers (n=3 placebo, 3 iron) had baseline sTfR>8.0 mg/dL. This suggests normal erythropoiesis in all subjects and impaired tissue iron status in only a small fraction of our sample at baseline.

Calculation of TBI via the sTfR:sFer index (12) may be preferable to sFer alone to assess iron status and the need for supplementation in athletes. An advantage of using the index is that, unlike sFer alone, it is sensitive to a wider range of body iron status, as it includes a measure of iron-deficient erythropoiesis as well as or tissue iron status. In IDNA athletes, only those with elevated sTfR values showed performance that was responsive to iron supplementation (5, 13, 14). In the current study, 5 rowers began the study with TBI<0 mg/kg (3 of whom had high sTfR, as mentioned previously). At the end of the study, only one of those rowers had a TBI <0 mg/kg, however, 13 rowers (n=8 placebo, 5 iron) had decreased TBI (range: -0.2 to -3.12 mg/kg), suggesting that the iron status of some subjects deteriorated after six weeks of training.

There is a negative correlation between iron supply and tissue iron deficiency. When there is increased iron supply with the same demand at the tissue level (sTfR),

tissues will produce fewer receptors for transferrin and sTfR will decrease. None of the iron status indicators used in this study were correlated with physical performance variables in the RCT (see *Chapter 7*). Hgb, the largest reserve of functional iron, has been associated with VO_2peak and blood lactate concentration, however, in our study, Hgb was not associated with lactate concentration.

Despite the absence of anemia in our study, a significant increase in Hgb was observed between the midpoint and end of the trial in all subjects ($+0.28 \pm 0.63$ g/dL, $p=0.02$). This could be due to rowers' hematological adaptation to training that has been previously reported (15, 16), or to hemoconcentration due to change in hydration levels during training. Although women in this study were not clinically anemic, criteria used to identify anemia (Hgb <12.0 g/dL) may be insufficient for female athletes. The potential for functional (non-clinical) anemia is much greater in this population due to intense training, which may further increase demand for O_2 -carrying capacity (Hgb) and functional tissue iron (sTfR).

On the other hand, researchers have shown increases in sTfR with muscle growth (17), and in the current study, after 6 weeks of training all rowers increased their FFM by 1.2 kg. Although we observed no correlation between change in FFM and change in sTfR, it is possible that this factor may have played a role in diminishing the effects of iron supplementation on this measure of iron status, and may be an explanation for why some rowers in the iron treatment group did not display a greater iron status response to treatment. In mixed effect multiple regression analyses, change in FFM was not a significant predictor ($\beta=0.10$, $p=0.72$) of change in sTfR, nor was treatment group ($\beta=-0.76$, $p=0.23$). The inclusion of an interaction term

(treatment group-by-change in FFM, Figure 9.4) was marginally significant ($\beta=0.73$, $p=0.19$), and inclusion of this interaction term greatly improved the effect of treatment group ($\beta=-1.6$, $p=0.08$) on change in sTfR in the regression model. Although insignificant, this is possibly an important interaction, and is consistent with previous findings from our lab showing that non-athletic IDNA women with the most FFM at baseline experienced the largest improvements in sTfR after 6 weeks of training and treatment (14).

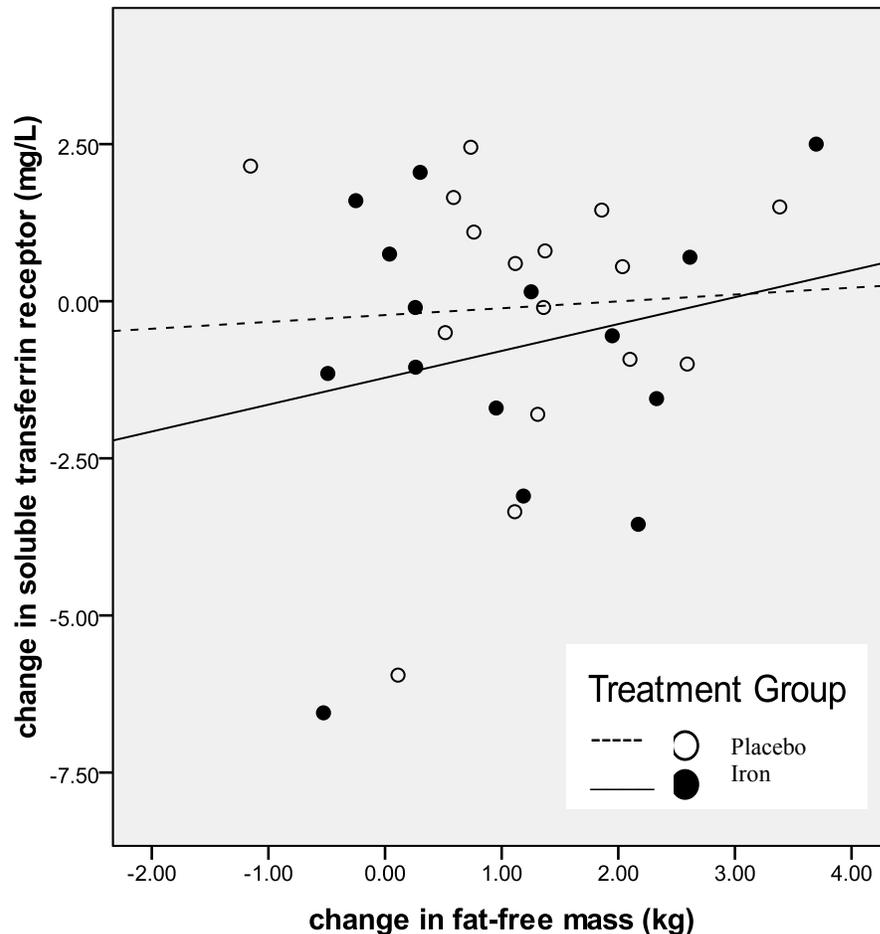


Figure 9.4. Relationship between change in fat-free mass (kg) and change in soluble transferrin receptor (mg/L) after 6 weeks of treatment ($n=31$)

Physical performance

Energetic efficiency: Given the findings that supplemental iron replenished rowers' iron stores, it is a logical next step to determine the effects of improved iron stores (via supplementation) on physical performance (energetic efficiency, lactate, VO₂ peak, and endurance capacity). In our cross-sectional analyses, we found that rowers with IDNA reported slower 2K personal records and had a lower VO₂peak and reduced energetic efficiency compared to rowers with normal iron status (*Chapter 4*). In the RCT analyses, although time to complete the 4K TT was unaffected by iron treatment, iron-supplemented rowers were able to significantly increase their energetic efficiency after controlling for baseline efficiency and supplement dose consumed. Furthermore, analysis of data from the subsample of rowers with baseline serum ferritin <20.0 µg/L showed that iron-supplementation clearly increased energetic efficiency by 1.3% after 6 weeks of training compared to the placebo group.

This means that after being supplemented with iron for 6 weeks, non-anemic rowers were able to perform the same workload at a lower energy cost (lower level of physical exertion = more energetically efficient). This finding is consistent with other iron supplementation studies of non-anemic women. Researchers have found that O₂ consumption (as % VO₂max) during an endurance test was significantly less after iron supplementation (-3%) and significantly greater in placebo (+3%) (18). Zhu & Haas showed that after 8 weeks iron supplementation, non-athletic women increased their efficiency by decreasing their energy expenditure by 5.1% (p=0.016) compared with women in the placebo group, and that this treatment effect on %VO₂peak was mediated by a change in Hgb (8). Hinton et al found that after a 4-week training

program imbedded in a 6-week iron supplementation trial, although both groups increased their efficiency (through training), there were no significant differences in total test efficiency between the treatment groups. However, the iron group decreased their O₂ consumption by 5% (as a %VO₂peak) during the last 5K of 15 K TT. (5). Another study of untrained IDNA Mexican women reported 5.2% greater efficiency during a cycle ergometer test after 6 weeks of supplementation with 18 mg of elemental iron (19). Subsequently, Hinton et al found that after 6 weeks of iron supplementation, recreational athletes' post-trial efficiency was significantly increased (+1.1%) compared to placebo (+0.7%), though the post-trial efficiency measure was not significantly different between the treatment groups (20). Additionally, the difference in energetic efficiency should not have been due to differences in psychological factors between the two treatment groups, as motivation scores measured throughout the trial were not significantly different between the two treatment groups at baseline and endpoint. Furthermore, in these studies we measured gross efficiency, which does not account for energy expended during and unloaded (0 watts) condition, termed net energetic efficiency. Measuring energy expenditure during an unloaded bout would have enabled us to calculate net efficiency, which may have been more sensitive to changes in iron status, and strengthened the relationships between iron status and performance.

Lactate response: In both the cross-sectional study and RCT, iron status/supplementation affected lactate response during the 4K TT. In the cross-sectional study, lactate concentrations at 2000m and 10-minutes post-test were significantly negatively correlated with log sFer ($r=-0.31$, $p=0.04$). Blood lactate

concentrations at pre-test, as well as 1000, 2000, 4000m (maximal), and 10-minutes post-test were significantly higher in rowers with IDNA compared to rowers with normal iron status (Figure 5.2), but after controlling for pre-test values, there were no significant differences between the two iron status groups. The higher pre-test lactate concentration in the IDNA group could be due to inadequate recovery from warming-up, or insufficiently warming-up prior to the start of the test compared to rowers with normal iron status. All rowers were encouraged to warm-up for at least 10-min prior to testing, but this was not monitored or recorded in this study.

At the end of the 6-week RCT, rowers in the iron treatment group had improved lactate response during the early stages of the 4K TT, regardless of baseline iron status (both IDNA and normal iron status). Rowers supplemented with iron showed a slower increase in lactate (10.3% lower, expressed as percent of maximal lactate, Figure 7.6) during the first half of the 4K TT (1000-2000m) compared to rowers in the placebo group. Supplementation may have improved lactate clearance, reduced lactate production, or both, and thus reduced utilization of lactate as fuel, during the first two legs of the TT. After the first 2000m, however, there was no difference between treatment groups until 5-minutes post-test, where rowers in the iron group recovered more quickly compared to those in the placebo group.

Decreased oxidative capacity (a consequence of IDNA) may affect glycolysis via the utilization of lactate as a fuel during exercise. When the rate of lactate produced is greater than the rate of clearance, lactate accumulates in the body (21). This accumulation of lactate causes fatigue and inhibits muscle contraction, leading to impaired performance during continuous endurance exercise. Due to their greater

lactate accumulation or decreased clearance early in the TT, IDNA rowers were utilizing their anaerobic energy pathways earlier than were the rowers with normal iron status, which is inherently less energetically efficient than using aerobic pathways.

Although many researchers have found no effect of iron supplementation on lactate concentration during exercise in IDNA women (5, 22-25), results from the current study are similar to those of Zhu and Haas who found that IDNA women supplemented with iron showed a slower rise in lactate concentration during first leg of 15K TT (5 K mark), as well as an inverse association between lactate concentration at the 5 K (first third of 15 K TT) and Hgb, even in marginal iron deficiency (8). Even in the absence of frank anemia (Hgb <12.0 g/dL), impaired O₂ transport capacity due to IDNA affects lactate metabolism, resulting in impaired oxidative metabolism, and ultimately increased reliance on anaerobic metabolism to produce energy (greater lactate production at an earlier stage of exercise). In a state of IDNA, lactate metabolism may be directly affected, resulting in the prevention or slowing of lactate clearance (26, 27).

The lack of treatment effect on lactate measured after the first half of the 4K TT could be due to our study being under-powered (small sample size, poor compliance), but is likely due to the fact that towards the end of the TT, lactate concentration was reaching a steady state, as well as its maximum tolerable limit, as the TT was performed at a fixed WR. Margin for improvement by iron supplementation was likely very small after the first 2000m, and was likely insensitive to supplementation.

VO₂peak : In the cross-sectional study, after controlling for fat-free mass, there was a difference in VO₂peak between rowers with IDNA and those with normal iron status only in the “low” training group. There was no difference in VO₂peak between IDNA and normal iron status in the group of rowers that reported training harder at the beginning of the season. We did not observe an effect of change in iron status on VO₂peak in the RCT. Rowers randomized into the trial who were IDNA at baseline were no different from rowers with normal iron status with regards to baseline performance, or endpoint performance. However, on average rowers with IDNA at baseline tended to have lower VO₂peaks at endpoint, regardless of treatment assignment (p=0.08). Among the rowers with IDNA at baseline, there were no differences in endpoint or change in VO₂peak between the two treatment groups.

In addition to our RCT being under-powered to demonstrate an effect on VO₂peak (small sample size and poor compliance), one explanation for the discrepancy in VO₂peak findings between the two studies may be training (as discussed above, *Internal Validity*). VO₂peak is highly dependent on training, and is predictive of endurance performance. Training increases mitochondrial density and number, as well as free fatty acid oxidation, leading to increased O₂ utilization capacity during exercise (28). Less highly-trained rowers, then, would have fewer mitochondria (meaning less metabolic potential to use O₂). We expected to see greater improvements in performance in rowers with IDNA at baseline compared to those with normal iron status at baseline. The iron status of IDNA rowers had a

greater potential to benefit from iron supplementation (as discussed above, *Plausibility*).

Although Hgb concentration is an important determinant of O₂ consumption, we found no significant correlations between Hgb and VO₂peak, probably because no rowers included in both the cross-sectional and RCT were anemic. In the subgroup of rowers with IDNA at baseline, however, change in VO₂peak was significantly associated with change in Hgb between weeks 3 and 6 of the RCT ($r=0.51$, $p=0.05$). This implicates Hgb as one of the determinants of VO₂peak in female athletes with IDNA despite absence of anemia. This could be due to the prevalence of functional (sub-clinical) anemia and the inadequacy of the generic Hgb cut-off to classify anemia in this population. We did not find a positive association between change in sFer (body iron stores) and change in VO₂peak in our RCT, but sFer may be indirectly involved in the utilization of O₂ via iron-containing enzymes of the tri-carboxylic acid cycle (TCA) cycle and electron transport chain (ETC).

Additionally, we may not have found an effect of iron treatment on VO₂peak in the RCT because there may be a non-linear relationship between Hgb, sFer, and VO₂peak. There may be threshold levels of sFer or Hgb, especially in athletes, and when either (or both) indicator is above or below these threshold levels, a linear positive relationship with VO₂peak emerges. There were, however, no differences in the ranges of sFer and Hgb in the two studies, which weakens this argument.

Rowers in our study represented a wide range of training and fitness levels (NCAA Divisions I and III, teams as well as an intramural club rowing team). Though training sessions across the five schools were comparable in terms of activities,

session duration and intensity differed widely across schools, as well as within-schools (between-rowers). As mentioned previously, we attempted to control for training and fitness differences between schools and seasons by using a mixed effects linear multiple regression model, but this method may not have accounted for all unmeasured confounders between schools or training seasons. In the RCT, our randomization was successful in that both treatment groups were no different in any measured baseline characteristic, iron status, or performance outcome. The discrepancy in findings between the two studies may also be due to smaller sample size of IDNA rowers in the intervention study (n=24 IDNA compared to 16 in the RCT).

Endurance capacity: Although rowers supplemented with iron did not improve their time to complete a 4K TT, studies have shown improved TT performance in IDNA non-athletic women after iron supplementation (5). Other researchers using time to exhaustion protocols did not observe an effect of iron status (18, 22, 25). This inconsistency between studies is likely due to methodological limitations of endurance testing. The exercise protocol (eg. TT vs time to exhaustion) needs to be sensitive enough to detect a difference in performance due to iron status, as well as adequately explore differences in energy metabolism. The time to exhaustion protocol is not the best measure of athletic performance in endurance athletes for several reasons. The test becomes too long, especially for highly-trained athletes, and consequently motivation becomes a major factor affecting the outcome of the test (29). Furthermore, this protocol does not mimic training or competitive event performance. The 4K fixed length time trial (TT) was used in this study as a measure of endurance

performance because it best represents rowers' training and on-water race performance (races last <30 min, short-duration, high-intensity). The 4K TT protocol also allows us to study proxies of oxidative metabolism (VO_2 , energetic efficiency, lactate) throughout the test, during which iron plays an important role, as mentioned previously. We most likely did not see a supplement or iron status effect on TT performance due to the over-riding influence of heavy training in many of these athletes, which decreased their margin for improvement with iron supplementation or change in iron status.

Training quality

In our cross-sectional study, rowers with IDNA at the beginning of a season reported training 10 minutes less per day compared to rowers with normal iron status. In the RCT, we observed that rowers supplemented with iron reported higher ratings of training session intensity, concentration, speed and stress throughout the trial. To adequately show the effect of iron supplementation or change in iron status on training, the measure of training needs to be sensitive enough to show difference between groups of iron status or treatments. In the cross-sectional study and the RCT, the session-RPE method was used to quantify training. Although this method has been used with various groups of athletes, and has been validated in our lab during rowing training (*Appendix 1*), it is possible that it inadequately captures the intermittent and varied nature of collegiate rowers' training activities. Much of rowing training revolves around repeated short bouts of high-intensity effort with intermittent rest (either on a rowing ergometer or in a boat on the water). It may have

been more difficult for rowers to adequately gauge and recall the intensity of an entire 2-hour workout if bouts were short with adequate rest between bouts.

In the supplementation trial, exploratory analyses led us to create a training quality score (sum of training intensity, motivation, concentration, discomfort, speed, and stress) which was then used in mixed effects multiple regression analyses. After controlling for training quality at baseline, iron supplementation resulted in a significantly greater improvement in training quality score at the end of 6 weeks compared to the placebo group. Differences in training quality were seen as early as two weeks into the study (see *Chapter 8*, Figure 8.3), and persisted throughout the study. The significant difference in training quality at two weeks was too early for the iron to have an effect, especially given the low dose that was being consumed as a result of poor compliance (refer back to Table 9.1). This difference at the two-week time point could be attributed to any unmeasured differences between the two groups. Differences in training quality were again significant at 6 weeks, which would be a reasonable amount of time for the iron treatment to affect training.

Although the effect of iron supplementation on training quality has not been previously studied, training itself has been implicated in changes in sFer (16, 16, 18, 30-37). A common explanation for changes in sFer include the mobilization of iron from stores to tissues to support Hgb and RBC synthesis needed in response to high training volume and/or intensity during the early stages of training. As adaptation to training occurs, hemodilution and erythropoiesis normalize, given adequate iron intake.

Another explanation is related to the inflammation of training, resulting in inflation of sFer as an acute phase protein, and/or the effect of training on the iron

regulatory protein, hepcidin. A few studies in active individuals have shown an increase in hepcidin, and consequent decrease in iron status, with intense training (38), but others have reported no effect of training on hepcidin activity (39). Liu et al attempted to identify the mechanism underlying the effects of training intensity on iron status and iron absorption using an animal model (40). Rats were randomly assigned to different training groups for 10 weeks: no training, moderate training, or strenuous training, and hepcidin mRNA expression, FPN 1, and DMT 1 were examined. Body iron status in the moderate-training group was maintained, as evidenced by higher DMT-1 and FPN1 and lower hepcidin mRNA expression compared to the sedentary and strenuous training groups. This study suggests that moderate exercise actually improved iron status. These results are contrary to the same group's most recent report of lower DMT1 and FPN1 and *up*-regulation of hepcidin mRNA expression in an exercised group of rats compared to a sedentary group (41). Rats' performance was not measured in either of these studies. Possible reasons for the discrepancy between the two studies include the length of training (10 weeks vs 5 weeks), as well as the intensity of training (moderate in the former vs various progressive intensity in the latter). The latter finding is more plausible, given the inflammation of training effect. Hepcidin was not measured in the current study of female rowers, but remains an important area of future research.

Recommendations to athletes, coaches, and collegiate athletic programs

Based on the literature review, data, and discussion presented in this thesis, active/training females are more vulnerable to IDNA, and increased iron intake via

supplementation and/or dietary means is recommended for these individuals. Due to the inflammation of training and its effects on sFer values, as well as on acute hepcidin secretion and iron absorption, athletes who are intensely training may benefit from iron supplementation, despite maintaining normal iron status. However, concerns regarding unregulated iron dosage and iron overload (pro-oxidant) should be considered, thus iron supplementation should not be initiated without proper determination of iron status, including markers of inflammation. There are currently no standards for the evaluation of iron status of female college athletes, but the American Dietetic Association and the American College of Sports Medicine suggest that female athletes' iron status should be periodically screened (42). A survey of NCAA Division I schools found that not only is iron status screening *not* a routine practice, but there is much variability in diagnostic and treatment criteria used between schools (43).

Given the roles that iron plays in exercise, accurately determining iron status in female endurance athletes is critical. At the beginning of the training season, female endurance athletes should be screened for iron deficiency and depletion with and without anemia using Hgb and sFer cut-off of 20.0 $\mu\text{g/L}$ to identify iron depletion before it leads to anemia or frank iron deficiency, thus reducing the adverse affects that iron depletion may have on their training and performance. After identification, anemic and IDNA athletes should be provided with treatment (e.g. low dose of supplemental iron, 18 mg) and/or nutrition counseling as necessary. Iron status and supplement compliance of anemic and IDNA athletes should be serially monitored throughout the training season to assure sufficient dietary iron intakes, and prevent

further decrements in iron status with training. Additionally, coaches should take an active role in monitoring training quality over the season, and recommend health status screening as needed.

Various supplements are employed by athletes to improve athletic performance (44). Surveys of female college athletes show that more than 50% use some type of supplement, iron or iron-containing multivitamins among the most popular (45). Also, college coaches routinely provide or recommend supplementation (46). With the general rise in supplement use, nutrition education targeted at both coaches and athletes on the use of traditional and non-traditional supplements is warranted. Nutrition education interventions targeted to college athletes have been successful in both increasing nutrition knowledge, as well as improving dietary intake (47, 48).

Future research

Strategies to easily screen and improve iron status may be useful for female endurance athletes at the beginning of a training season because data suggest that iron status of very active women may decrease with increased levels of physical training over time (16, 49-51). Studies focused on the implementation of screening policies for female athletes at both the high school and collegiate levels would help prevent ID, IDA and IDNA in this population and may confer benefits to other parts of their lives beyond athletics. Future research should examine the prevalence, risk factors, and mechanisms of IDNA in female athletes, including plasma markers of hemolysis, accurate measures of athletes' menstrual blood loss, dietary iron intake/absorption, as well as other markers of iron metabolism and inflammation, such as hepcidin, IL-6,

and IRP. Indicators of oxidative capacity (e.g. iron-dependent oxidative enzymes in muscle) are also needed to better control for potential confounders and investigate the mechanism by which IDNA alters energy metabolism and affects endurance performance.

Future studies should employ objective methods of training assessment, such as measured heart rate and accelerometry to quantify energy expenditure, training intensity, frequency, and duration during the training season. These methods may be more sensitive to changes in iron status and to the effects of iron supplementation. Also, further examining the relationship between iron and aspects of training such as motivation and concentration would be helpful to elucidate the mechanisms behind the relationships between iron, training, and performance in athletes.

It would also be important to focus on ways to improve supplement compliance in training athletes in order to confer maximum benefit to athletes' iron status. This could include using iron-fortified food products or beverages (versus capsules), which may be more acceptable to athletes and could improve compliance (39, 52, 53). Also, dose frequency is another way to examine compliance (e.g. a high dose of iron less-frequent weekly dose vs. lower dose daily). Some studies have shown improved iron stores, as well as better compliance with a more infrequent dose of iron compared to daily supplementation (9, 9, 54, 55) while others have not (56).

Defining optimal ferritin levels in female athletes would be helpful in order to target those who would benefit most from supplementation. Furthermore, it is important to know how that ferritin level specific to female athletes can be enhanced and maintained most effectively (supplementation vs dietary intervention). Though it

has been shown that physical exercise can lead to a decline in body iron status, we do not know the level of dietary (or supplemental) iron that would be sufficient to meet the increased demand of chronic endurance training. Defining iron requirements in female athletes is another area that needs more research. Supplementing female endurance athletes with various dosages of elemental iron (e.g. 15, 30, 50, or 100 mg/d) during a training season is needed to answer this question.

Finally, future research should investigate different methods to successfully deliver sports nutrition education to both athletes and coaches. To facilitate and maintain positive behavior change, design and implementation of education and screening interventions should be multi-level and multi-disciplinary, involving athletes, coaching staff, athletic trainers and the athletic department, campus health services (including dietitians), and food and dining services. College athletes would especially benefit from interventions promoting self-efficacy (57, 58), specifically in the areas of supplement compliance, menu planning, cooking demonstrations, and making healthy eating choices while traveling to and from competitions.

Conclusions

The studies described in this dissertation have shown that there are important relationships between iron status, training and performance in female rowers. Analyses of plausibility and probability have been discussed, along with the strengths and limitations of the cross-sectional study and RCT. Non-significant findings for major outcome measures of change in iron status, training, and performance have been

explained, and are due in-part to small sample size and poor compliance with the iron treatment intervention.

Our RCT has shown that IDNA in female collegiate rowers affects physical performance as measured by gross energetic efficiency (RCT). This indicates that IDNA increases rowers' exertion and energy cost to do the same load of work, and that iron supplementation enhanced some, but not all indicators of rowers' adaptation to training. In addition, rowers supplemented with iron showed a potentially beneficial delayed lactate response during the first half of and early recovery from a 4K TT, indicating that lactate metabolism is affected by iron depletion in the absence of frank anemia. These results are important for female endurance athletes whose dietary patterns and physical training levels increase their risk of IDNA, and suggest that iron supplementation may maximize the benefits of endurance training.

Despite our findings, many questions remain to be answered to complete the picture of how iron status affects training and performance in female athletes. More research is needed to understand the functional consequences of IDNA in training female endurance athletes before either supplemental and/or dietary iron intake recommendations can be made for this population. Future research should be focused on the hematological changes during endurance training and their effects on iron status, as well as strategies to easily screen and treat female athletes with poor iron status, before training and performance are adversely affected. Optimal cut-offs of iron status indicators for female athletes, as well as optimal iron doses to prevent and/or treat sub-clinical iron deficiency (IDNA) in female athletes should be explored.

Results from this study add to the evidence that iron status is an important issue facing female endurance athletes at the beginning of a training season. We have shown that IDNA is prevalent among female collegiate rowers. We have also shown that rowers who consumed ~15 mg elemental iron/d improved their iron stores (sFer) during training (after controlling for baseline sFer), and that those rowers with the lowest iron stores at baseline benefitted the most from iron supplementation, which adds to the growing body of evidence that iron supplementation improves the iron status of active women, which may ultimately impact training and physical performance.

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APPENDICES

Appendix 1: Validation of training measure

VALIDATION OF TRAINING MEASURE: QUANTIFICATION OF ROWERS' TRAINING LOAD USING THE SESSION RPE METHOD

Introduction

Several methods have been used to monitor athletes' training load, including heart rate (HR), measurement of total physical activity energy expenditure (EE), subjective rating of perceived exertion (RPE), and training impulses (TRIMPs). TRIMPs are arbitrary units of stress calculated from training session intensity and time used to quantify total training load (1). While determining training load in individual events (e.g. long-distance running) is reasonably straightforward (2), techniques for monitoring the training load of individuals during intermittent team activities are lacking. Rowing is a team sport with these intermittent patterns of varying intensity, and training is often monitored and analyzed via individual ergometer "scores" and video analysis. Session rating of perceived exertion (sessionRPE) has been used to quantify a variety of training activities (3-6), but has not been tested in rowers. The objective of this descriptive study was to compare measured heart rate (HR) with subjective ratings of intensity (RPE) to quantify training load in healthy, well-trained female collegiate rowers both in the lab and on the water. This analysis enabled us to validate the training load metric (sessionRPE) used in our cross-sectional study and iron supplementation trial investigating the effect of iron on performance and training.

Methods

Recruitment of subjects: This study was approved by the Institutional Review Boards of Cornell University and Syracuse University. Subjects were recruited during the conditioning phases of their competitive rowing seasons (fall 2009, spring 2010),

as well as prior to a pre-season training trip. All varsity and second-semester novice female rowers were eligible to participate in the screening if greater than 18 years of age, non-smoking, and were regularly training on the rowing team. Training activities during this time included on-water and ergometer rowing of varying intensity, as well as general aerobic conditioning (cycling, running).

Cornell University's women's rowing coach was notified of the study, and aided in recruitment by passing along the information verbally at the beginning of a practice session. Additionally, undergraduate research assistants aided in the recruitment of rowers via email flyers and word-of-mouth. Rowers from Syracuse University were recruited while already participating in the iron supplementation trial. All rowers provided written informed voluntary consent prior to participating in the study. Health and demographic data from each rower was collected to ensure subjects' ability to safely participate in athletic training and physical exercise testing protocols, and a medical screening (NCAA-required) prior to our study excluded all athletes not healthy enough to participate in their rowing team training (current, acute or chronic illness, severe asthma, musculoskeletal problems, etc). Subjects were given information from fitness and body composition tests as the benefit of participation.

Physical performance testing methods: Body weight and height were measured with standard procedures and equipment (7). Body fat and composition was assessed via air-displacement plethysmography (BodPod, Life Measurement, Inc, Concord, CA). Physical fitness and endurance performance was assessed using a rowing ergometer (Concept2, Morrisville, VT) equipped with a digital readout monitor (PM2), displaying work (watts, W), stroke rate (spm), distance (m), and elapsed time

(min:sec). A computerized metabolic cart (TrueMax 2400, ParvoMedics, Salt Lake City, Utah) was used to measure VO_2 and other physiological measures during all testing. Concentrations of O_2 and CO_2 in expired air were analyzed with gas analyzers (which are routinely calibrated with gases of known O_2 and CO_2 concentration). Respiratory volume (V_E) was measured with a respiratory pneumotachograph (Fitness Instrument Technologies, Farmingdale, NY) through a two-way breathing valve (Hans Rudolph, Kansas City, MO).

Energy expenditure (EE) was assessed via indirect calorimetry during exercise testing using a standard protocol that measured expired gases for V_E , VO_2 , VCO_2 , and respiratory exchange ratio (RER), all monitored continuously throughout testing (8). Heart rate (HR, Polar FS2, Polar Electro, Inc, Lake Success, NY) was also continuously monitored throughout testing. Cadence (spm) and work rate (WR, watts (W) resistance) were monitored and recorded every 30 seconds.

Blood samples were obtained by finger or ear punctures immediately before and after testing. Blood lactate concentrations were determined by the Lactate Pro analyzer (FaCT Canada, Quesnel, British Columbia, Canada) (9), which we have concluded to be valid and accurate against an enzymatic assay ($r=0.64$, $p<0.001$, Sigma Diagnostics, St. Louis, MO), as is consistent with the peer-reviewed literature (10-13).

Subjects were instructed to not consume food or beverages other than water, or to perform any strenuous physical activities 2 h prior to testing. To control for the effects of dietary intake and hydration status, subjects were instructed to record all food and fluid intake 3d prior to testing, as well as the day of exercise testing

(Appendix 7). Subjects had the opportunity to warm-up for at least 10 min prior to all testing.

VO₂peak: Rowers performed three tests in the lab. The first was a pre-test done in order to acclimate subjects to testing protocol and laboratory procedures, as well as to establish a VO₂peak. VO₂peak was determined by a modified version of the maximum aerobic power (MAP) test, which is a ramped protocol used by rowing coaches to assess training progress (14).

Rowers' MAP in split-time was converted into watts ($W = 2.8/\text{pace per } 500 \text{ m}^3$), and the test began 100 W below the predicted maximum. Each stage of the test lasted 90s, with a 10s "gear-up" period between each stage (Appendix 12). Every 90 s, the rower was asked to increase her WR by 20 W, until she was no longer able to maintain the WR. This test was designed to last between 8-10 min. VO₂peak was identified as the highest VO₂ value achieved, and was confirmed by at least one of the following: 1) VO₂ increased by <150 ml/min with an increase in WR; 2) RER >1.10; or 3) HRmax was within 10 beats of age-predicted maximum (220-age) (15). A 15-min cool-down period followed testing at a self-selected WR, and HR was monitored for 10 min post-test. Blood sampling for lactate was collected pre- and post test, as well as at 5- and 10-min post-test. Complete test time was about 45 min (10-15 min to acclimate to equipment; 10 min for actual testing; 15 min cool-down). Participants were able to stop the test at any time, and the investigator was able to stop the test at any time (equipment malfunction; subject symptoms of severe fatigue).

From the VO₂peak test, HR (x)-VO₂(y) calibration curves were plotted for each subject. These curves were used to predict VO₂ and EE from on-water training

HR data (outside of the lab). HR (bpm) was converted to predicted VO_2 (based on each individual's VO_2 peak HR- VO_2 calibration curve), and then to EE (5 kcal per L O_2). This EE was summed and divided by 4 (4-15-sec epochs/min) to calculate total min-by-min EE during each training session.

Rowing workout simulations on the ergometer: Two ergometer workouts modeled after on-water training were performed in the lab. VO_2/CO_2 , HR, etc was monitored during the tests as described above. After a self-selected warm-up, subjects were asked to perform both a routine “Hard” training session, and a routine “Easy” training session, each session was separated by at least 3d. Rowers chose the order in which the tests will be administered (“Easy” vs “Hard”), as dictated by their training schedule.

The “Easy” workout simulation began with 2 min pulling at 50% pressure (18-20 spm), followed by one minute pulling at 75% pressure (22-24 spm), followed by one minute pulling at full (100%) pressure (open stroke rating, range 26-32 spm). This was followed by one minute of drills (e.g. “pause at the finish,” etc), as would be performed in a boat on the water. This 5-minute sequence was repeated 4 times (4 x 5 min), after which rowers were asked to “paddle back to the dock,” as they would during an on-water practice in the boat. The entire “Easy” protocol lasted about 25 min. For the “Hard” training session protocol, rowers were asked to perform a “stroke rate pyramid:” row for 1-minute on followed by 2-minutes paddle at stroke rates of 26-28-28-30-30-32 (open) spm, followed by a “paddle back to the dock.” This “Hard” protocol lasted about 25 min. HR data from both the “Easy” and “Hard” training

session simulations were then plotted across time along with WR to examine the intermittent patterns of rowing training.

Monitoring of training: Subjects were asked to maintain and record their normal on-water rowing training load daily, as well as to wear a HR monitor on their chest (Polar E-series, Polar Electro USA, Lake Success, NY) during all training sessions. HR (bpm) was recorded in 15-sec intervals and data from each on-water training session was plotted across time to examine the intermittent pattern of rowing training, and these patterns were compared with those simulated in the lab.

Intensity ratings after laboratory testing and rowing training regimen outside of the lab was quantified via detailed training and activity records (Appendix 11). Questions in the daily log addressed sleep and nap duration and quality, soreness and fatigue, and training or activity frequency, intensity, time, and type. Questions were presented in the format of a Visual Analog Scale (VAS) (16). Subjects were asked to rate each question by placing a solid vertical line on a 100mm scale anchored by opposing descriptors (see Figure A.1). All VAS questions were “scored” by measuring the rating with a ruler (mm). The session-RPE method (3) was then used to quantify daily training load. VAS Intensity score for each training session was multiplied by training session duration (time).

Daily Log Instructions

This training log should be completed on a ***daily basis*** for the next 7 days. Initial use of this log may take up to 5 minutes/day. For some questions, please rate each factor, as you feel ***today*** by placing a ***solid vertical line*** on the scale.

Example: Happy: How happy do you feel right now?

Not at all happy _____ | _____ Extremely happy

Figure A.1 . Visual Analog Scale (VAS) instructions and format of training log

Analysis of training data: Training sessions (both in and outside of the lab) were categorized according to four major types (steady-state and drills, strength and power, endurance, and mixed). HR data was used to determine training intensity based on HR <75%, 75-85%, and >85% of subjects' HR_{max} (as determined in the VO₂peak test). The average HR and the peak HR for each training session was noted, and the training intensity distribution was quantified from the minutes spent in each %HR_{max} zone for each individual training session (17). The average time in each zone for all sessions was then determined. Ratings of intensity for each session were divided into the three zones, and training zone identification based on HR was compared with that sessionRPE method.

Data Analysis: All data are presented as the mean \pm sd. Training intensity ratings and physical performance data collected during the laboratory training simulations were compared using a t-test, and Pearson's correlations and linear regression analysis were used to examine relationships between TRIMP and the sessionRPE methods of calculating training load. A p-value <0.05 was considered statistically significant.

Results

Physical performance during the VO_2 peak test is shown in Table A.1. Rowers ($n=11$) were 20 ± 0.8 years old, weighed 72.4 ± 10.2 kg ($23.5\pm 3.0\%$ BF), and were 178.2 ± 6.8 cm tall. HR- VO_2 calibration curves were calculated for seven of these rowers ($R^2=0.72\pm 0.2$), and an example of a typical curve is presented in Figure A.2.

Table A.1. Physical performance during the VO_2 peak test ($n=11$)

	Mean \pm SD
VO_2 peak, ml/kg/min	47.7 \pm 4.8
VO_2 peak, L/min	3.4 \pm 0.5
RERmax	1.1 \pm 0.07
HRmax, bpm	188.6 \pm 10.6
WRmax, W	249.3 \pm 27.3
Maximal lactate, mmol/L	10.8 \pm 1.9
Slope of HR- VO_2 curve, L O_2 /min	0.51 \pm 0.37 (0.03-1.2)
Intercept of HR- VO_2 curve	75.2 \pm 56.2 (2.1-162.6)

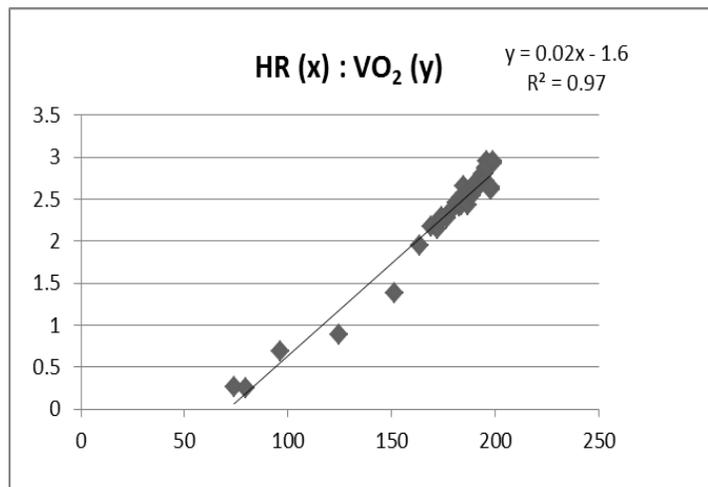


Figure A.2. Graphic example of typical subject HR- VO_2 calibration curve

Seven rowers completed the simulated workouts in the laboratory. Data from the “Easy” and “Hard” workout simulations is presented in Table A.2. The heart rate and work rate patterns for all of the subjects during the easy day training session were similar within each test type (see Figure A.3 A and B). The “easy” simulation elicited a significantly different physiological response from the “Hard” simulation, and rowers rated the intensity of the “Easy” session significantly lower than the “Hard” session ($p=0.04$).

Table A.2. Laboratory workout simulations, mean \pm SD (range)

	Easy	Hard	p-value
Mean VO ₂ (ml/kg/min)	32.1 \pm 2.7 (28.05-36.2)	33.5 \pm 4.6 (28.3-39.5)	0.26
Peak VO ₂ (ml/kg/min)	44.3 \pm 3.8 (39.1-50.4)	47.8 \pm 5.9 (40.4-56.3)	0.01
Mean HR (bpm)	151.7 \pm 15.1 (134.5-174.5)	160.4 \pm 14.6 (144.8-175.9)	0.06
Peak HR (bpm)	180.6 \pm 11.4 (168.3-196.9)	189.7 \pm 8.4 (178.7-198.5)	0.04
Mean work rate (w)	138.0 \pm 19.7 (111.1-162)	152.1 \pm 22.0 (127.2-180.7)	<0.001
Peak work rate (w)	242.8 \pm 39.6 (192-293)	298.2 \pm 25.5 (264-319)	0.01
Post-test blood lactate concentration (mmol/L)	2.96 \pm 1.5 (1-5.2)	6.9 \pm 2.3 (4.4-9.8)	0.01
Total EE (kcal)	286.9 \pm 42.0 (225.9-349)	272.5 \pm 48.2 (216-347.3)	0.29
Rate EE (kcal/min)	11.2 \pm 2.4 (7.8-13.7)	12.0 \pm 2.2 (9.8-15.8)	0.41
VAS intensity rating (mm)	40.2 \pm 18.0 (25-63)	79.4 \pm 8.6 (64-90.4)	0.04

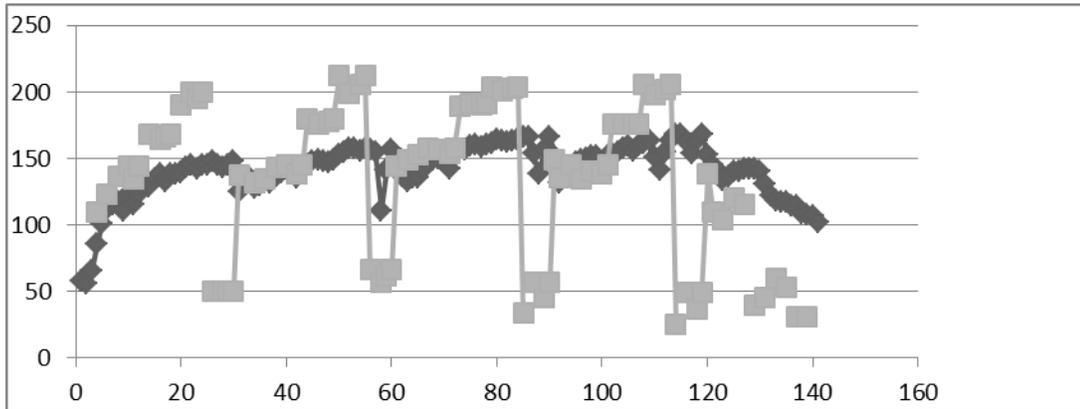


Figure A.3 A. Graphic example "Easy" workout simulation

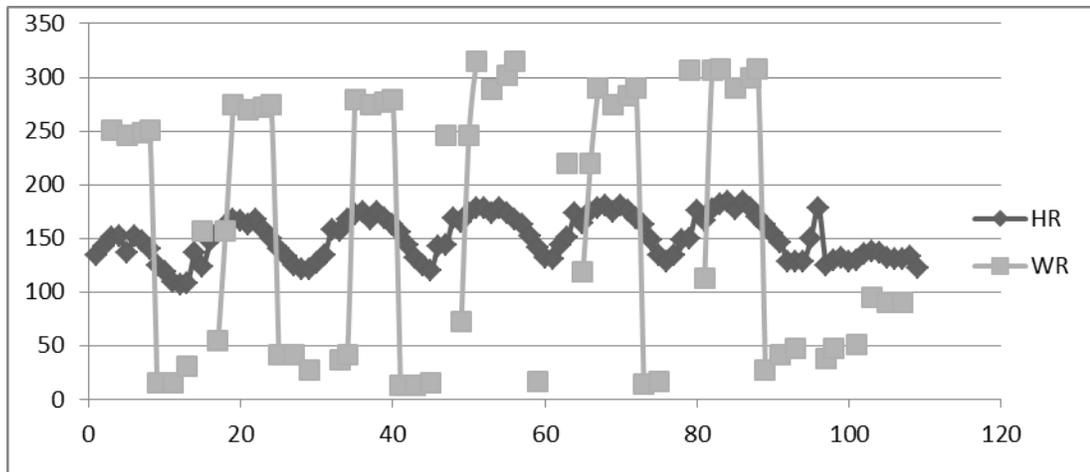


Figure A.3 B. Graphic example "Hard" workout simulation

Summary of training sessions outside of the lab are presented in Table A.3. Eighty-one training sessions were recorded and analyzed (7 ± 3 sessions per rower; range: 2-12 sessions).

Table A.3. Training sessions outside of lab

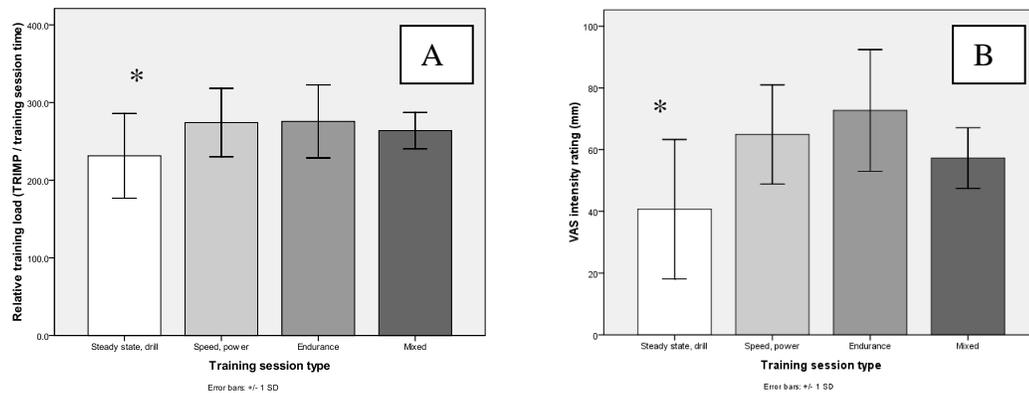
	Mean \pm SD
Mean training duration (min/session)	102.1 \pm 33.9
Training HR (bpm)	118.9 \pm 12.3
Peak HR during training (bpm)	176.15.3
Percent time in HR zone (%)	
Zone 1: <55%	30.9 \pm 16.1
Zone 2: 55-64%	22.8 \pm 8.0
Zone 3: 65-74%	19.4 \pm 6.4
Zone 4: 75-84%	14.0 \pm 7.7
Zone 5: 85-94%	10.0 \pm 7.8
Zone 6: >95%	2.5 \pm 4.0
Mean training load (summated HR zone method)	263.0 \pm 109.4
Mean training load (sessionRPE method)	6029.8 \pm 3830.0

Training load varied by session type, and this data is presented in Table A.4. There were no significant differences in training time or absolute TRIMP among the four session types. However, relative TRIMP during steady-state/drills training was significantly lower than during the strength/power ($p=0.02$) and endurance training ($p<0.001$, Figure A.4 A). VAS intensity ratings during steady-state/drills training were also significantly lower than during the strength/power ($p=0.009$) and endurance training ($p<0.001$, Figure A.4 B).

Table A.4. Training loads according to session type

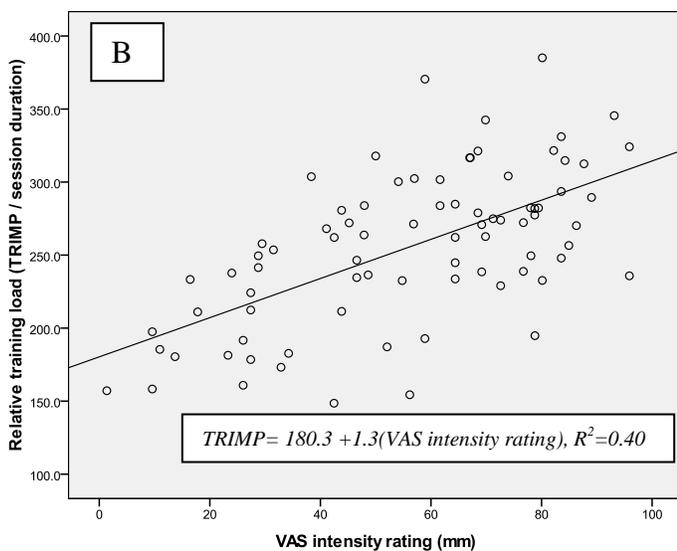
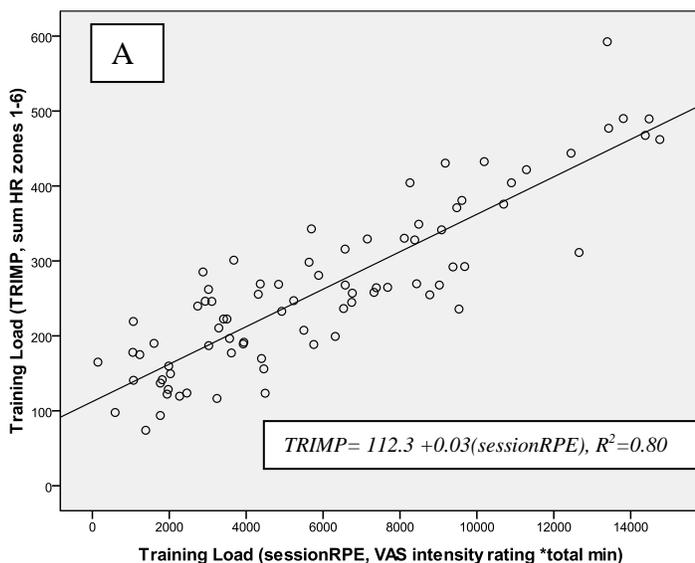
Training session type	Number of sessions (n)	Session duration (min)	VAS intensity rating (mm)	TRIMP (summed HR)
Steady-state, drills	35	93.1±29.8	40.7±22.6	215.5±86.6
Strength and power	20	97.5±30.7	64.9±16.1	265.9±94.7
Endurance	21	115.5±35.2	72.7±19.7	321.4±115.3
Mixed	5	126.8±47.2	57.3±9.8	338.5±147.4

Figure A.4. A and B. Training load distribution quantified by VAS intensity rating (A) and TRIMP (B), according to session type. *Significantly different from speed/power and endurance sessions.



There was a significant correlation between training loads calculated using the TRIMP (HR summation) and sessionRPE methods ($r=0.88$, $p<0.001$). After accounting for time in both methods, there was still a significant correlation between VAS intensity rating and relative TRIMP ($r=0.61$, $p<0.001$). Linear regression analysis revealed that both sessionRPE and VAS intensity rating were significant predictors of TRIMP (Figure A.5 A) and relative TRIMP (Figure A.5 B), respectively ($p<0.001$).

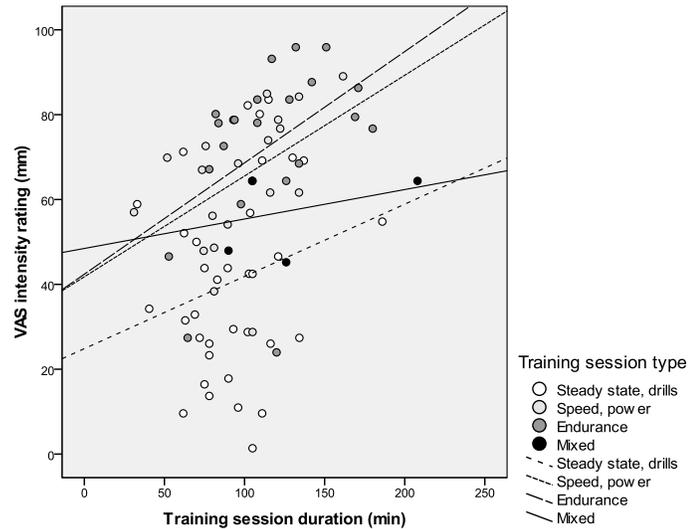
Figure A.5 A and B. Relationship between training load calculated using the absolute (A) and relative (B) sessionRPE and TRIMP methods



Training session type was not a significant predictor of relative TRIMP, nor was there a significant time-by-session type interaction. However, training session

duration ($\beta=0.19$, $p=0.01$) and training session type ($\beta=9.8$, $p<0.001$, $R^2=0.30$) were both significant predictors of VAS intensity ratings (Figure A.6).

Figure A.6. Relationship between training session duration and VAS intensity rating according to training session type



Discussion

The present study examined the validity and application of the sessionRPE method for quantifying training loads of female rowers during several types of rowing training activities. Our results are consistent with previous investigations. We found good agreement between the session RPE method using our VAS-intensity rating and the summated heart rate zone method (17) during both in-laboratory simulations of training, and rowing training outside of the lab. Given the importance of high-intensity training to performance, the intermittent nature of rowing, and difficulties associated with collecting HR data during rowing training, the sessionRPE method provides a valid alternative to monitoring training load in this group of athletes.

Differentiation of training session types and relation to sessionRPE has not been previously reported. Rowers in this study trained, on average, six times per

week, and there were often two separate training sessions per day of varying intensity. The four session types differentiated in this study were the combined result of subject report, analysis of HR data, and familiarization with goals of rowing training (RJD, SKK, KAA, EKC). Training sessions consisting of steady state exercise and drills were related to a technical focus, not intensity. During these sessions, there was very little pulling at full pressure (maximal workload) and HR remained <75% of HRmax (see “Easy” workout simulation). Strength/power and endurance sessions consisted of several bouts or intervals of short (2K or less), full-pressure (maximal workload) pieces. Intensity during these sessions was high, despite the rest time between intervals (see “Hard” workout simulation).

Data from this study also show that rowers in our study spent much of their training time working below 75% of their maximal HR, which is consistent with previous findings (4). This is due largely to the fact that the time spent within each HR zone or as a percent of maximal HR, included sedentary/non-active time during training (e.g. waiting for instruction, paddling back to the start, etc). The intermittent nature of rowing training likely skewed the amount of time spent below a certain threshold, and we controlled for this time factor in the analysis of relative TRIMP and VAS intensity rating.

The limitations of this study include the absence of measured resting HR for each subject, which limits our ability to examine each individual’s HR response to training and predict EE outside of the lab. Also, our small sample size was small and we collected a limited number of training sessions. It would be useful to quantify

training load using the two methods in several rowers on the same team, during the same workout sessions to examine the reliability within- and between-subjects.

Conclusions: Within the limitations of this study, we found the sessionRPE method to be a valid metric of training load. Additionally, the method's convenience, cost-effectiveness, and non-invasiveness makes it a feasible option for researchers and coaches to quantify and monitor training load. From a practical standpoint, application of the sessionRPE method would allow coaches to evaluate and compare adaptation to training within- and between individuals.

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Appendix 2. Voluntary informed consent

Cross-sectional study consent form

Investigator: Diane M. DellaValle, MS, RD & Dr. Jere D. Haas, PhD

Purpose of the Study: The purpose of this research is to investigate the association between iron status, fitness, and physical activity level in women.

Procedures: Participation in this research will first entail completing a health history questionnaire and having a blood sample collected by a trained phlebotomist. The blood sample will be used to determine your iron status only and will not be used for any other purpose. Based on the results of the blood test you may then be asked to perform a maximal and sub-maximal treadmill and sport-specific ergometer (row, swim) tests, having a body composition test done by two non-invasive techniques, wear 3 activity monitors and maintain a physical activity record for 6 days. In addition, we may ask you to return for a second body composition test once you have finished wearing the activity monitors for 6 assigned days.

The body composition tests will consist of two parts. The first test consists of having skinfold measurements performed which require a fatfold to be measured at five separate sites (tricep, thigh, hip, bicep, and beneath your shoulder blade) using skinfold calipers. The second part will use a device called the Bod Pod. This will entail wearing a one-piece swimming suit and sitting inside a closed chamber. For part of the test you will be wearing a nose clip and be breathing through a tube so we can measure your lung volume.

Maximal aerobic capacity (VO₂max) will be assessed. The VO₂max tests on the rowing ergometer will begin with a five minute self-selected warm-up; the work load will then be set back 5- 20W levels from your fastest 2K erg split time for 1.5 minutes. Every 1.5 minutes will be increased by 20 W until you can no longer continue. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort. We may stop the test at any time because of signs of fatigue, symptoms you may experience, or equipment malfunction. During the test you will also be asked to wear a nose-clip and mouthpiece, which will be supported by a head device. For the test you will be breathing only through your mouth into a hose that is connected to a metabolic cart for the measurement of oxygen uptake. You will also be asked to wear a heart rate monitor that will be strapped to your chest.

The endurance test and will also be on the rowing ergometer and will consist of a 4K time trial. After a 5-min self-selected warm-up, you will be rowing at a workload equivalent to 85% of your maximal level reached during the maximal exercise test. The test will be conducted in 1000 m intervals, after every 1000 m, there will a 60-sec break during which we will collect a small blood sample from your finger and/or ear lobe. At the last 400 m of the test, you will be asked to sprint to the finish, as you

would in a boat race. During this test you will be fitted with a mouthpiece, nose clip and heart rate monitor as described above. The endurance test will be performed on a separate day as the maximal exercise test, but the test must be completed within 7 days of each other.

During both the VO₂max and endurance tests, we will collect small blood samples from your finger or earlobe to determine your blood lactate levels.

Outside of the lab, you will be asked to wear 1 activity monitor and maintain a physical activity record/training log for 7 assigned days. The monitor is worn on the waist band during waking hours (except for bathing and swimming), and you will be instructed on how to remove it before you go to bed and where to place it when you wake up in the morning.

This study requires a great deal of preparation and includes a considerable expense to us. Thus, if you cannot keep your phlebotomy appointment or other testing appointments, we ask that you give us at least 24 hours notice so that we can reschedule a time for you. Since each subject can have a great impact on the study, it is important that you carefully read through each questionnaire and complete all of the questions. If you feel that this is not possible, please do not join the study.

Discomforts and Risks: This study requires a small sample of blood (10 mL) to be collected from each subject. The risk this entails includes fainting, nausea, and dizziness in some persons. Our phlebotomist is trained to deal with these situations, should they occur. Some minor annoyances may include slight soreness and bruising on your arm due to the blood collection, and on your fingers due to the finger pricks to collect samples for lactate analysis. There are no major risks involved in filling out the questionnaires and daily logs. To protect subject identity, all information obtained is filed according to an assigned subject number in a locked filing cabinet. Your name and assigned subject number will be kept in a separate locked filing cabinet that only the principle investigator will have access to.

Any discomforts due to exercise testing are not uncommon to those participating in training and competition in endurance sporting activities. Healthy individuals rarely experience the following risks while performing moderate or maximal exercise: abnormal blood pressure responses, musculo-skeletal injuries, dizziness, difficulty in breathing, and in rare instances heart attack or death.

Benefits: You will receive your blood work results after your sample has been analyzed. Also, if you are recognized as anemic, you will be notified so you can be treated appropriately. In addition, you will receive information about you body composition, fitness level, and physical activity level. This information will be useful to your training program.

Duration/Time: The health history questionnaire will take approximately 45 minutes to complete. The blood sample will take approximately 5 minutes to collect. The maximal exercise test will take 45 minutes to complete, the sub-maximal test will take 1 hour, the body composition tests will take 30 minutes, and wearing the activity monitor and maintaining the physical activity record will be performed for 7 assigned days.

Compensation: You will not be monetarily compensated for your participation in this study.

Contact Information: If you have questions at any time about the study or the procedures, (or you experience adverse effects as a result of participating in this study,) you may contact the Investigator, Diane M DellaValle at (607) 229-3683, 213 Savage Hall, Ithaca, NY 14853. If you have questions about your rights as a participant, contact the Cornell University Office of Research Integrity and Assurance, Institutional Review Board (IRB) for Human Participants at (607) 255-5138, or access their website at <http://www.irb.cornell.edu/>. You may also report your concerns or complaints anonymously through [Ethicspoint](#) or by calling toll free at 1-866-293-3077. Ethicspoint is an independent organization that serves as a liaison between the University and the person bringing the complaint so that anonymity can be ensured. You may also contact the Syracuse University IRB with any questions about your rights as a participant, or with any questions, concerns, or complaints you wish to address to someone other than the investigator, or in the event that you cannot reach the investigator, or for any research-related injuries (ph: 315-443-3013, email: orip@syr.edu, web: <http://orip.syr.edu>).

Right to Ask Questions and to Withdraw

You are free to decide whether or not to participate in this study and are free to withdraw from the study at any time. If you choose to withdraw from the study at any time your present and future relationship with Cornell University and Syracuse University will not be adversely affected. Before you sign this form, please ask questions about any aspects of the study, which are unclear to you. In addition you will be given a copy of this form to keep for your records.

Consent: By signing this paper, I am indicating that I understand and agree to take part in this research study, and I am indicating that I am 18 years of age or older.

Your signature

Date

Researcher's signature

Date

This consent form will be kept by the researcher for at least three years beyond the end of the study and was approved by the IRB on (06/10/2008)

(RCT Consent Form)

Investigators: Diane M. DellaValle, MS, RD & Dr. Jere D. Haas, PhD

Purpose of the Study: The purpose of this research is to investigate the association between iron status, fitness, and physical activity level in women.

Procedures: A blood sample will be collected by a trained phlebotomist to determine your iron status only and will not be used for any other purpose. Blood samples will be collected at weeks 4 and 8 during the 8-week study. You will be randomized to a Placebo (no nutritional value) or supplement (iron) group for 8 weeks. Neither subjects nor investigators involved in monitoring and measuring subjects will know who received which treatment until after all hypotheses have been tested. You will be given and instructed to consume 2 capsules every day for 8 weeks. Minor gastrointestinal discomfort is a potential side-effect of iron supplementation. You are to consume the capsules with citrus juice to enhance iron absorption, and with meals to reduce gastrointestinal side effects. You are to avoid consumption of any other multivitamin/mineral supplements during the 8 week study period. You are required to record capsule consumption, dietary intake, and keep records of medication and other permitted supplement intake, illness, menstrual status, GI symptoms, physical activity and training, and any musculoskeletal problems in a daily log.

Body composition will be measured at weeks 4 and 8. The body composition tests will consist of two parts. The first test consists of having skinfold measurements performed which require a fat-fold to be measured at five separate sites (tricep, thigh, hip, bicep, and beneath your shoulder blade) using skinfold calipers. The second part will use a device called the Bod Pod. This will entail wearing a one-piece swimming suit and sitting inside a closed chamber. For part of the test you will be wearing a nose clip and be breathing through a tube so we can measure your lung volume.

Maximal aerobic capacity (VO₂max) will be assessed at 8 weeks. The VO₂max tests on the rowing ergometer will begin with a five minute self-selected warm-up; the work load will then be set back 5- 20W levels from your fastest 2K erg split time for 1.5 minutes. Every 1.5 minutes will be increased by 20 W until you can no longer continue. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort. We may stop the test at any time because of signs of fatigue, symptoms you may experience, or equipment malfunction. During the test you will also be asked to wear a nose-clip and mouthpiece, which will be supported by a head device. For the test you will be breathing only through your mouth into a hose that is connected to a metabolic cart for the measurement of oxygen uptake. You will also be asked to wear a heart rate monitor that will be strapped to your chest.

The endurance test will be conducted at week 8, and will also be on the rowing ergometer and will consist of a 4K time trial. After a 5-min self-selected warm-up, you will be rowing at a workload equivalent to 85% of your maximal level reached

during the maximal exercise test. The test will be conducted in 1000 m intervals, after every 1000 m, there will a 60-sec break during which we will collect a small blood sample from your finger and/or ear lobe. At the last 400 m of the test, you will be asked to sprint to the finish, as you would in a boat race. During this test you will be fitted with a mouthpiece, nose clip and heart rate monitor as described above. The endurance test will be performed on a separate day as the maximal exercise test, but the test must be completed within 7 days of each other. During both the VO₂max and endurance tests, we will collect small blood samples from the finger or earlobe to determine your blood lactate levels.

Outside of the lab, you will be asked to wear 1 activity monitor worn on the waist band and maintain a physical activity record/training log for 1 week at a time, every other week throughout the 8 weeks. The waist-mounted monitor is worn during waking hours (except for bathing and swimming), and you will be instructed on how to remove it before you go to bed and where to place it when you wake up in the morning. This study requires a great deal of preparation and includes a considerable expense to us. Thus, if you cannot keep your phlebotomy and other testing appointments, we ask that you give us at least 24 hours notice so that we can reschedule a time for you. Since each subject can have a great impact on the study, it is important that you carefully read through each questionnaire and complete all of the questions. If you feel that this is not possible, please do not join the study.

Discomforts and Risks: This study requires a small sample of blood (10 mL) to be collected from each subject. The risk this entails includes fainting, nausea, and dizziness in some persons. Our phlebotomist is trained to deal with these situations, should they occur. Some minor annoyances may include slight soreness and bruising on your arm due to the blood collection, and on your fingers or ear lobes due to the pricks to collect samples for lactate analysis. There are no major risks involved in filling out the questionnaires and daily logs. To protect subject identity, all information obtained is filed according to an assigned subject number in a locked filing cabinet. Your name and assigned subject number will be kept in a separate locked filing cabinet that only the principle investigator will have access to. Minor gastrointestinal discomfort is a potential side-effect of iron supplementation.

Any discomforts due to exercise testing are not uncommon to those participating in training and competition in endurance sporting activities. Healthy individuals rarely experience the following risks while performing moderate or maximal exercise: abnormal blood pressure responses, musculo-skeletal injuries, dizziness, difficulty in breathing, and in rare instances heart attack or death.

Benefits: You will receive your blood work results after your sample has been analyzed. Also, if you are recognized as anemic, you will be notified so you can be treated appropriately. In addition, you will receive information about you body composition, fitness level, and physical activity level. All information give to you will benefit your training program.

Duration/Time: The health history questionnaire will take approximately 45 minutes to complete. The blood sample will take approximately 5 minutes to collect. The maximal exercise test will take 30 minutes to complete, the submaximal/endurance test will take 60 min, and the body composition tests will take 15 minutes. The activity monitor will be worn for 1 week at a time every other week, and maintaining the physical activity record will be performed daily throughout the 8 weeks.

Compensation: You will not be monetarily compensated for your participation in this study.

Contact Information: If you have questions at any time about the study or the procedures, (or you experience adverse effects as a result of participating in this study,) you may contact the Investigator, Diane M DellaValle at (607) 229-3683, 213 Savage Hall, Ithaca, NY 14853. If you have questions about your rights as a participant, contact the Cornell University Office of Research Integrity and Assurance, Institutional Review Board (IRB) for Human Participants at (607) 255-5138, or access their website at <http://www.irb.cornell.edu/>. You may also report your concerns or complaints anonymously through [Ethicspoint](#) or by calling toll free at 1-866-293-3077. Ethicspoint is an independent organization that serves as a liaison between the University and the person bringing the complaint so that anonymity can be ensured. You may also contact the Syracuse University IRB with any questions about your rights as a participant, or with any questions, concerns, or complaints you wish to address to someone other than the investigator, or in the event that you cannot reach the investigator, or for any research-related injuries (ph: 315-443-3013, email: orip@syr.edu, web: <http://orip.syr.edu>).

Right to Ask Questions and to Withdraw

You are free to decide whether or not to participate in this study and are free to withdraw from the study at any time. If you choose to withdraw from the study at any time your present and future relationship with Cornell University or Syracuse University will not be adversely affected. Before you sign this form, please ask questions about any aspects of the study, which are unclear to you. In addition you will be given a copy of this form to keep for your records. -----

Consent: By signing this paper, I am indicating that I understand and agree to take part in this research study, and I am indicating that I am 18 years of age or older.

Your signature

Date

Researcher's signature

Date

This consent form will be kept by the researcher for at least three years beyond the end of the study and was approved by the IRB on (06/10/2008)

Appendix 3 Health and demographic questionnaire

1. Health & Demographics Questionnaire, pg1

Age: _____ Date of Birth: _____ Sex: Female

Height: _____ Weight: _____

Are you currently under a physician's care? Yes No

Do you smoke: Yes No If yes, how many cigarettes per day? _____

Do you drink alcohol: Yes No If Yes, frequency and amount of alcohol: _____

Ethnicity (please check only one):

- HISPANIC OR LATINO
 NOT HISPANIC OR LATINO

Race (please check only one):

- AMERICAN INDIAN/ALASKAN NATIVE WHITE
 ASIAN HAWAIIAN/PACIFIC ISLANDER
 BLACK OR AFRICAN AMERICAN

Eating Behavior

What time do you usually eat the following meals?

Breakfast: _____ Dinner: _____
Lunch: _____ Snack(s): _____

Are there foods you don't eat because they are not good for you or disagree with you?

Yes No

If yes, what foods? _____

Are there any foods you don't eat because of medication you are on? Yes No

If yes, what foods? _____

Are there any foods you make it a point to eat because you feel they are good for your health?

Yes No

If yes, what foods? _____

Are there any foods you don't eat because they are difficult to chew? Yes No

If yes, what foods? _____

Are you a vegetarian? Yes No

Are you a vegan? Yes No

Do you eat:

Fish Yes No

Chicken/poultry Yes No

Eggs Yes No

Beef Yes No

Milk Yes No

Health & Demographics Questionnaire, pg2

Medications:

Are you presently taking medication (over the counter and/or prescription)? Yes No

If yes, please specify drug(s), when last taken, and dose:

Have you taken any analgesics (aspirin, Tylenol, ibuprofen, etc) in the past month? Yes

No

If Yes, give the name: _____, When last taken: _____ and dose: _____

Have you taken any pain relievers in the past 3 months? Yes No

If Yes, give the name: _____, When last taken: _____ and dose: _____

Do you take any medications for depression or sleeping difficulty?

Currently, Yes Type and Brand: _____

Currently, No When Stopped: _____ Type and Brand used before: _____

Never

Do you take antihistamines? Yes No If Yes, please specify: _____

Are you taking oral contraceptives? Yes No If Yes, please specify: _____

Are you taking any vitamin, mineral, or herbal supplements? Yes No

If Yes, please specify item, when last taken, and dose:

Have you ever received radiation therapy? Yes No

Have you ever received chemotherapy? Yes No

Weight History:

Current weight: _____

Highest past adult weight (*excluding pregnancy*): _____

When did this occur? _____

Lowest past adult weight: _____ When did this occur? _____

Have you experienced any weight change in the last 6 months? Yes No

If yes, did you gain or lose? _____ How much? _____

When did this weight change occur? _____

Health & Demographics Questionnaire, pg3

Medical and Surgical History Overview

1) Acute problems (please list, explain if necessary):

2) Recurrent problems (please list, explain if necessary):

3) Chronic problems (please list, explain if necessary):

4) Did you have a fever, inflammatory or any infectious diseases in the past 3 months?

Yes No If Yes, please explain what it was and how long ago:

5) History of fainting when blood samples are taken? Yes No

6) Exercise-induced asthma? Yes No

7) Any allergies Yes No

If Yes, please explain:

8) History of digestive problems? Yes No

If Yes, please explain:

9) Glucose intolerance? Yes No

10) Abnormal blood lipid profile? Yes No

11) Recurrent dislocations? Yes No

12) Recent (past 3 months) musculoskeletal injuries? Yes No

If Yes, please
explain: _____

13) Past orthopedic surgery? Yes No

If Yes, please
explain: _____

14) Other major surgery? Yes No

If Yes, please
explain: _____

Health & Demographics Questionnaire, pg4

Do you have, or have you had any of the following?

- | | |
|---|--|
| <input type="checkbox"/> High blood pressure | <input type="checkbox"/> Diabetes |
| <input type="checkbox"/> Heart trouble | <input type="checkbox"/> Ulcers (of the digestive system) |
| <input type="checkbox"/> Thyroid or other glandular disorders | <input type="checkbox"/> Other stomach or intestinal disorders |
| <input type="checkbox"/> Liver disease | <input type="checkbox"/> Kidney disease |
| <input type="checkbox"/> Anemia | <input type="checkbox"/> Depression |
| <input type="checkbox"/> Cancer | <input type="checkbox"/> Respiratory illness (asthma, etc.) |
| <input type="checkbox"/> Angina pectoris | <input type="checkbox"/> Myocardial infarction |
| <input type="checkbox"/> ECG disorder | |
| <input type="checkbox"/> Heart murmur | |
| <input type="checkbox"/> Cardiac rhythm abnormalities | |
| <input type="checkbox"/> Other, please specify _____ | |

Have you ever been diagnosed as anemic or iron deficient? Yes No

If Yes, which type? Please check the following and indicate the date of the most recent diagnosis.

- Sickle cell anemia or thalassemia
- Pernicious anemia
- Aplastic anemia
- Hemolytic anemia
- Iron-deficiency only
- Hypoplastic anemia
- Iron-deficiency anemia
- Other, please specify _____

Have you ever been diagnosed, or have a family history of iron overload disease (hemochromatosis)? Yes No

Do you have any of the following eating related problems? Please check all those that apply:

- | | |
|--|---------------------------------------|
| <input type="checkbox"/> Sore mouth | <input type="checkbox"/> Nausea |
| <input type="checkbox"/> Swallowing problems | <input type="checkbox"/> Vomiting |
| <input type="checkbox"/> Chewing problems | <input type="checkbox"/> Diarrhea |
| <input type="checkbox"/> Choking problems | <input type="checkbox"/> Constipation |
| <input type="checkbox"/> Food allergies:_____ | |
| <input type="checkbox"/> Salivation problems | |
| <input type="checkbox"/> Other, please specify | |

Have you ever been diagnosed/treated for an eating disorder(s)/ disordered eating behaviors? Yes No

If Yes, please explain type and date(s):_____

Are you currently on any kind of special diet? Yes No

If yes, what kind (low-salt, low-fat, etc.):_____

Health & Demographics Questionnaire, pg5

Menstrual Status

- At what age did you first menstruate?_____
- What was the date of your last menstrual period?_____
- In the previous 12 months, has your menstrual cycle been (*please check only one*):
 Regular (normal cycles of approximately equal length)
 Irregular (amenorrhea; absence of periods; irregular periods; missed cycles, cycles of varying length, marked changes in flow; when did the abnormality start?)
Please explain:
 I did not menstruate in the last 12 months
- How many days does your menstrual cycle last (from the beginning of the menstrual period to the beginning of the next period)?
- Your menstrual blood flow is (circle one): Low Normal High
- How many pads/tampons do you use per day on the peak-flow day: _____ pads/tampons
- How many days of heavy flow do you experience:_____ days of heavy flow
- Do you use an intrauterine device (IUD)? Yes No
If Yes, how long have you been using it?_____

9) Have you taken any hormones (birth control pills, Depo-Provera®, hormone replacement therapy, etc.) in the past year?

10) Have you given birth in the past 12 months? Yes No

11) Are you planning to become pregnant within the next 12 months? Yes No

Blood Loss

1) Do you donate blood regularly? Yes No

2) When was the last time you donated blood: _____

3) How much blood did you donate: _____

4) Did you lose blood for reasons other than menstruation in the past 3 months? Yes No

If Yes, how much: _____ and When: _____

Appendix 4 Female athlete screening tool (FAST):Instructions: Please circle the answer that applies best to each of the numbered statements. **Exercise** = Physical activity lasting ≥ 20 min; **Practice** = Scheduled time allotted by coach to work as a team or individually in order to improve performance; **Training** = Intense physical activity. The goal is to improve fitness level in order to perform optimally.

1. I participate in additional physical activity ≥ 20 min in length on days that I have practice or competition.	Frequently	Sometimes	Rarely	Never
2. If I cannot exercise, I find myself worrying that I will gain weight.	Frequently	Sometimes	Rarely	Never
3. I believe that most female athletes have some form of disordered eating habits.	Strongly agree	Agree	Disagree	Strongly disagree
4. During training , I control my fat and calorie intake carefully.	Frequently	Sometimes	Rarely	Never
5. I do not eat foods that have more than 3 g of fat.	Strongly agree	Agree	Disagree	Strongly disagree
6. My performance would improve if I lost weight.	Strongly agree	Agree	Disagree	Strongly disagree
7. If I got on the scale tomorrow and gained 2 pounds, I would practice or exercise harder or longer than usual.	Frequently	Sometimes	Rarely	Never
8. I weigh myself:	Daily	≥ 2 times per week	Weekly	Monthly
9. If I choose to exercise on the day of a competition, I exercise for:	≥ 2 hours	45 min – 1 hour	30-45 min	<30 min
10. If I know that I will be consuming alcoholic beverages, I will skip meals on that day or on the following day.	Frequently	Sometimes	Rarely	Never
11. I feel guilty if I choose fried foods for a meal.	Frequently	Sometimes	Rarely	Never
12. If I were to be injured, I would still exercise even if I was instructed not to do so by my athletic trainer, physician, or coach.	Strongly agree	Agree	Disagree	Strongly disagree
13. I take dietary and/or herbal supplements in order to increase my metabolism and/or to assist in burning fat.	Frequently	Sometimes	Rarely	Never
14. I am concerned about my percent body fat.	Frequently	Sometimes	Rarely	Never
15. Being an athlete, I am very conscious about consuming adequate calories and nutrients on a daily basis.	Frequently	Sometimes	Rarely	Never
16. I am worried that if I were to gain weight my performance would decline.	Strongly agree	Agree	Disagree	Strongly disagree
17. I think that being thin is associated with winning.	Strongly agree	Agree	Disagree	Strongly disagree
18. I train intensely for my sport so that I will not gain weight.	Frequently	Sometimes	Rarely	Never
19. During the season, I choose to exercise on my one day off from practice or competition.	Frequently	Sometimes	Rarely	Never
20. My friends tell me that I am thin, but I feel fat.	Frequently	Sometimes	Rarely	Never
21. I feel uncomfortable eating around others.	Frequently	Sometimes	Rarely	Never
22. I limit the amount of carbohydrates that I eat.	Frequently	Sometimes	Rarely	Never
23. I try to lose weight to please others.	Frequently	Sometimes	Rarely	Never
24. If I were unable to compete in my sport I would not feel good about myself.	Strongly agree	Agree	Disagree	Strongly disagree
25. If I were injured and unable to exercise, I would restrict my calorie intake.	Strongly agree	Agree	Disagree	Strongly disagree

Appendix 5 Eating inventory (EI): Read each of the following 36 statements carefully. If you agree with the statement, or feel that it is true as applied to you, answer true by circling the “T”. If you disagree with the statement, or feel that it is false as applied to you, answer false by circling the “F”.

1. When I smell a freshly baked pizza, I find it very difficult to keep from eating, even if I have just finished a meal.	T	F	19. Being with someone who is eating often makes me hungry enough to eat also.	T	F
2. I usually eat too much at social occasions, like parties and picnics.	T	F	20. When I feel sad or blue, I often overeat.	T	F
3. I am usually so hungry that I eat more than three times a day.	T	F	21. I enjoy eating too much to spoil it by counting calories, counting grams of fat, or watching my weight.	T	F
4. When I have eaten my quota of calories or fat, I am usually good about not eating any more.	T	F	22. When I see a real delicacy, I often get so hungry that I have to eat it right away.	T	F
5. Dieting is so hard for me because I just get too hungry.	T	F	23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	T	F
6. I deliberately take small helpings as a means of controlling my weight.	T	F	24. I get so hungry that my stomach often seems like a bottomless pit.	T	F
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	T	F	25. My weight has hardly changed at all in the last ten years.	T	F
8. Since I am often hungry, I sometimes wish that an expert would tell me that I have had enough to eat or that I can have some more.	T	F	26. I am always hungry, so it is hard for me to stop eating before I finish the food on my plate.	T	F
9. When I feel anxious, I find myself eating.	T	F	27. When I feel lonely, I console myself by eating.	T	F
10. Life is too short to worry about dieting.	T	F	28. I consciously hold back at meals in order not to gain weight.	T	F
11. Since my weight goes up and down, I have gone on reducing diets more than once.	T	F	29. I sometimes get very hungry late in the evening or at night.	T	F
12. I often feel so hungry that I just have to eat something.	T	F	30. I eat anything I want, any time I want.	T	F
13. When I am with someone who is overeating, I usually overeat too.	T	F	31. Without even thinking about it, I take a long time to eat.	T	F
14. I have a pretty good idea of the number of calories or grams of fat in common foods.	T	F	32. I count calories or grams of fat as a conscious means of controlling my weight.	T	F
15. Sometimes when I start eating, I just can't seem to stop.	T	F	33. I do not eat some foods because they make me fat.	T	F
16. It is not difficult for me to leave something on my plate.	T	F	34. I am always hungry enough to eat at any time.	T	F
17. At certain times of the day, I get hungry because I have gotten used to eating then.	T	F	35. I pay a great deal of attention to changes in my figure.	T	F
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	T	F	36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high-calorie foods.	T	F

37. **How often are you dieting in a conscious effort to control your weight?**

1	2	3	4
rarely	sometimes	usually	always

38. **Would a weight fluctuation of five pounds affect the way you live your life?**

1	2	3	4
not at all	slightly	moderately	very much

39. **How often do you feel hungry?**

1	2	3	4
only at meal times	sometimes between meals	often between meals	almost always

40. **Do your feelings of guilt about overeating help you to control your food intake?**

1	2	3	4
never	rarely	often	always

41. **How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?**

1	2	3	4
easy	slightly difficult	moderately difficult	very difficult

42. **How conscious are you of what you are eating?**

1	2	3	4
not at all	slightly	moderately	extremely

43. **How frequently do you avoid buying a large amount of tempting foods?**

1	2	3	4
almost never	seldom	usually	almost always

44. **How likely are you to shop for low-calorie or low-fat foods?**

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

45. **Do you eat sensibly in front of others and splurge alone?**

1	2	3	4
never	rarely	often	always

46. **How likely are you to consciously eat slowly in order to cut down on how much you eat?**

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

47. **How frequently do you skip dessert because you are no longer hungry?**

1	2	3	4
almost never	seldom	at least once a week	almost every day

48. **How likely are you to consciously eat less than you want?**

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

49. **Do you go on eating binges even though you are not hungry?**

1	2	3	4
never	rarely	sometimes	at least once a week

50. **To what extent does this statement describe your eating behavior?**

“ I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow. ”

1	2	3	4
not like me	a little like me	pretty good description of me	describes me perfectly

51. On a scale of 1 to 6, where 1 means no restraint in eating (eat whatever you want, whenever you want it) and 6 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?

- 1 Eat whatever you want, whenever you want it
- 2 Usually eat whatever you want, whenever you want it
- 3 Often eat whatever you want, whenever you want it
- 4 Often limit food intake, but often “give in”
- 5 Usually limit food intake, rarely “give in”
- 6 Constantly limit food intake, never “give in”

Appendix 6 Food frequency questionnaire for dietary iron

Think about your most recent eating habits and how often you eat each of the following foods. Please mark one box for each food. Please write in other iron-fortified foods where there is space available if those foods you consume are not listed. Also, please write in vitamins/minerals, supplements and medications in the space available if what you consume is not listed (and check the frequency of these, as well).

	Preparation? Portion size?	Last 4 weeks		Each Week			Each Day			
		0x	1-3x	1x	2-4x	5-6x	1x	2-3x	4-5x	6+x
Meats										
Beef liver										
Chicken liver										
Pork liver										
Beef										
Pork										
Chicken										
Turkey										
Tuna										
Eggs										
Vegetables										
Spinach										
Green peas										
Broccoli										
Legumes (chickpeas, lentils, black beans)										
Nuts/Seeds										
Other dark greens:										
Fruits/Juices										
Prune juice										
Apricots										
Raisins										
Orange juice, regular										
Orange juice, extra C										
Orange juice, other fortified (Calcium, D, other)										
Fortified Breakfast Cereals*	Brand name? Portion size?									
Cream of wheat										
Raisin bran										
Special-K										
Total										

FFQ pg2. Fortified Breads*	Brand name? Portion size?	Last 4 weeks		Each Week			Each Day			
		0x	1-3x	1x	2-4x	5-6x	1x	2-3x	4-5x	6+x
White										
Wheat										
Whole Wheat										
Rye										
Multigrain										
Other										
Vitamin/Mineral Supplements	Dose? Brand name?									
Multi-vitamin										
Mega-Multi-vitamin										
Iron supplement										
Vitamin C										
Calcium										
Other:										
Other Supplements										
Fiber										
Other:										
Medications (please list)										

Appendix 7 Food record

**Food and Activity Diary
Instructions for Participants**

The following information will help you in keeping your food diaries, and is intended to add to the information provided to you by the members of the study staff.

1. On the first line, record your name and the date of the food/activity diary.
For example:

Name: Diane M. DellaValle

Date Completed: 06/02/2004

2. Record the following on your Diary sheet:
 - a. Time of the food item or meal eaten
 - b. Record H or C (Homemade or Commercial)
 - c. Record the Place where you ate the item or meal: home, restaurant (specify name), friend's house
 - d. Record the Amount of each item: see Common Serving Sizes guides to help estimate.
 - e. Description of the food: record each item on a separate line. If a food contains several items, such as a sandwich, list each ingredient on a separate line. Also, indicate how the food was prepared (Baked, fried, broiled, grilled, with or without skin)

For example:

Time	H or C	Place	Amount	Description of Food
8 AM	C	Home	1 c ¾ c ½ c	Raisin bran cereal 2% milk Orange juice, Tropicana, not from concentrate, some pulp
10.00 AM	C	Home	1	Fruit cocktail cup, Dole
12.30 PM	H	Home	1 2 slices ¼ c 1 t	Tuna fish sandwich Whole wheat bread Tuna, canned in water Hellman's mayonnaise,
			full-fat	
4.30 PM	C	Friend's house	2 leaves 1	Iceberg lettuce Fudgesicle
			1-12 oz can	Coca-cola
7.00 PM	C	Home	1/8 of 16"	Pizza, plain, DiGiorgno
	H		3 oz	Chicken, baked, with skin
8.30 PM	C	Home	½ c	Vanilla ice cream,
			Breyer's full-fat	

Appendix 8 Leisure-time physical activity (LTPA) and Rowing PR

These questions are about your physical activities (exercise, sports, physically active hobbies...) that you may do in your LEISURE time.

How often do you do **VIGOROUS** leisure-time physical activities for **AT LEAST 10 MINUTES** that cause **HEAVY** sweating or **LARGE** increases in breathing or heart rate? (e.g. running, cross-country skiing, cycling, basketball)

- 000 Never
- 001-995 _____ per day / week / month / year
- 996 Unable to do this type activity
- 999 Don't know

About how long do you do these vigorous leisure-time physical activities each time?

- 001-995 _____ minutes / hours
- 999 Don't know

How often do you do **LIGHT OR MODERATE LEISURE-TIME** physical activities for **AT LEAST 10 MINUTES** that cause **ONLY LIGHT** sweating or a **SLIGHT** to **MODERATE** increase in breathing or heart rate? (e.g., walking, easy cycling, tennis)

- 000 Never
- 001-995 _____ per day / week / month / year
- 996 Unable to do this type activity
- 999 Don't know

About how long do you do these light or moderate leisure-time physical activities each time?

- 001-995 _____ minutes / hours
- 999 Don't know

Do you have a job(s) other than student (circle one)? Yes No

If Yes, what is your job(s)? _____

How many hours per week do you work at each job?: _____

Fe & Training – Events – Crew

Please indicate your personal records (PRs) from last season (Fall08-Spring09) for the following:

How many years of rowing experience have you had?_____

When was the last time you were on an erg?_____

Best (PR) **2K Erg** Time:_____

Best (PR) **6 K Erg** Time:_____

Fastest (PR) **500m split** time (for a 2K):_____

Your **PRESENT** (what you can do **NOW**) 2 K Erg Time:_____

Your **PRESENT** (what you can do **NOW**) 500m split time (for a 2K):_____

Appendix 9 Iron status report

Date: _____

Dear Study Participant _____,

Here is a report of your iron status. There are 4 values we examined:

- 1) RBC (red blood cell count) – Red blood cells function in hemoglobin transport, which results in delivery of oxygen to the body tissues. Low values may indicate anemia, but the cause of the anemia may require further testing.
- 2) Hgb (hemoglobin) – Hemoglobin is the oxygen-carrying pigment of the RBCs. It is composed of amino acids that form a single protein called globin and a compound called heme. Heme contains iron atoms and the red pigment porphyrin. Low values indicate anemia, but the cause of the anemia may require further testing.
- 3) Hct (hematocrit) – Hematocrit is the percentage of RBC in a volume of whole blood. Low values may indicate anemia, the cause of which must be confirmed by further tests
- 4) Fer (ferritin) – Ferritin is a measure of iron stored in the liver. Low values accompanied by low Hgb indicate iron deficiency anemia.

Next to your values, I have included the range of normal values for your reference. Circled below is a description of your iron status.

Your Value	Normal Value Range
RBC _____	3.90 – 5.70 x10⁶/uL
Hgb _____	12.0 – 16.0 g/dL
Hct _____	35.0 – 47.0 %
Fer _____	12 – 150 ng/mL

Normal iron status: All of your iron status measures are within the normal ranges.

Anemia: Your Fer values are within the normal range. Your Hgb values are outside of the normal range. An iron supplement or multi-vitamin containing iron may be of benefit to you at this time. If you are regularly taking an iron supplement, you should consult with a physician to ensure that the dosage of iron you are taking is adequate, as well as to monitor your iron status.

Iron-deficiency with Anemia: Both your Fer and Hgb values are outside of the normal ranges. You should continue to monitor your Fer and Hgb values. An iron supplement or multi-vitamin containing iron may be of benefit to you at this time. If you are regularly taking an iron supplement, you should consult with a physician to ensure that the dosage of iron you are taking is adequate.

Iron-deficiency without Anemia: Your Fer values are outside of the normal range. Your Hgb is within the normal range. You should continue to monitor your Fer and Hgb values. An iron supplement or multi-vitamin containing iron may be of benefit to you at this time. If you are regularly taking an iron supplement, you should consult with a physician to ensure that the dosage of iron you are taking is adequate.

If you have any questions about this report, please contact me (Diane M. DellaValle, dd235@cornell.edu) for further information. Thanks so much again for your participation in our study!

Sincerely,

Appendix 10 Body composition and fitness test report

**Cornell University
Iron and Training Study Fall 2009
Body Composition & Fitness Testing Results**

First and foremost, we would like to thank you for participating in our study! We really appreciate your time and enthusiasm! We have compiled your Iron Status, Body Composition and Fitness Testing results in this summary profile.

If you have any questions regarding this information, please contact Diane M DellaValle, dd235@cornell.edu. Thanks again for all of your time! We have really enjoyed working with you!

Sincerely,

A handwritten signature in black ink, appearing to read "D. DellaValle". The signature is fluid and cursive, with a large initial "D" and a long, sweeping underline.

Diane M DellaValle, MS, RD, Graduate Student
Cornell University
Division of Nutritional Sciences

Iron and Training Study, Fall '09 Body Composition

Baseline **End-point**

Body Weight = _____ kg (_____ lb) **_____ kg (_____ lb)**
_____ %

Your weight in kilograms as measured in your bathing suit prior to the body composition measurements. Your “ideal weight” is best determined from your body composition rather than from “average weights”.

Body Height = _____ cm (_____ in) **_____ cm (_____ in)**
_____ %

Your height in centimeters as measured in your bathing suit prior to the body composition measurements.

Body Mass Index (BMI) = _____ kg/m² **_____ kg/m²**
_____ %

BMI (Body Mass Index) = wt/ht². This index is used to indirectly assess energy balance. BMI is based on assumption that weight corrected for height is correlated with body fatness & obesity.

A BMI of <18.5 is indicative of underweight status, which may be associated with malnutrition or disease. A BMI of 18.5-24.9 indicates normal weight status, associated with little health risk. A BMI 25.0-29.9 indicates overweight status, which may be associated with health problems when coupled with other weight- or cardiovascular-related conditions. A BMI >30 indicates obesity, which poses significant health risks. BMI does not distinguish between fat mass & fat-free mass (body composition), and tends to overestimate fatness in muscular individuals

Percent Body Fat = _____ % **_____ %**
_____ %

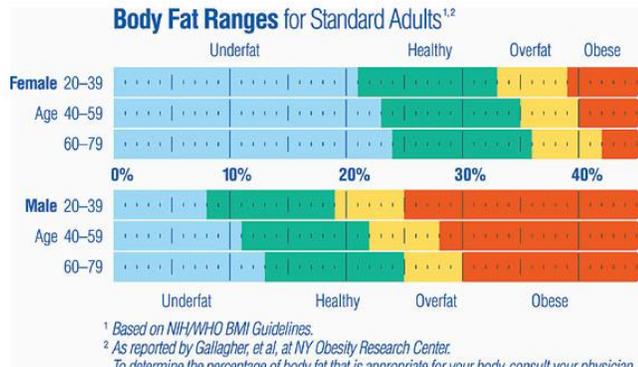
This value represents the percentage of your body that is composed of fat. Normal values for your age and gender are given in the chart below.

Lean Body Mass = _____ kg **_____ kg**
_____ %

This represents the weight of your lean tissues such as muscle and bone. This value should be preserved or increased in most people. It is undesirable to lose lean body mass in any weight loss program except in people who are morbidly obese.

Fat Weight = _____ kg **_____ kg**
_____ %

This is the weight of your body fat. This is the value we would like to lower in those people who need to lose weight.





Iron and Training Study, Fall '09
Fitness Assessment

Maximal Heart rate (HRmax) during exercise testing (beats per minute, bpm)

<i>Baseline</i>	<i>Endpoint</i>
<i>%Change</i>	
_____ (bpm)	_____ (bpm)
	_____ %

Five Heart Rate Fitness Zones

Zone	Ideal For	Benefit Desired	Intensity Level (% Maximum heart rate)
1 LITE	Light Exercise	Maintain Healthy Heart/Get Fit	50% - 60%
2 FAT	Weight Management	Lose Weight/ Burn Fat	60% - 70%
3 BASE	Aerobic Base Building	Increase Stamina Aerobic Endurance	70% - 80%
4 ANAR	Optimal Conditioning	Maintain Excellent Fitness Condition	80% - 90%
5 RED	Elite Athlete	Maintain Superb Athletic Condition	90% - 100%

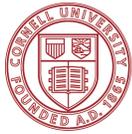
To calculate the percent of your HRmax to determine HR levels for training, first find your predicted HRmax, which is equal to **220 - your age in years**. Multiply that number (predicted HRmax) by the training level you desire. To find the **lower limit of your HR training range = HRmax*0.50**. To find the **upper limit of your HR training range = HRmax*0.90**.

Oxygen consumption (VO2) is also known as oxygen intake, oxygen utilization, oxygen uptake. This is the amount of O2 taken into the body and used in the cells of your body to produce energy. The ability to take up oxygen is dependent on how well your heart, lungs, blood, blood vessels, and muscle cells function. VO2 is a useful measure of aerobic fitness, and is represented in liters of oxygen per minute (l/min) or milliliters per kilogram per minute (ml/kg/min).

<i>Baseline</i>	<i>Endpoint</i>
<i>%Change</i>	
Your VO2 _{max} : _____ ml/kg/min	_____ ml/kg/min
	_____ %
_____ l/min	_____ l/min

VO2max is the maximal amount of oxygen the body can use during a given period of time, and is measured during a graded exercise test to fatigue during which the work load is progressively increased, which increases O2 consumption. VO2max represents the functional capacity of the cardio-respiratory system. In short, VO2 increases as exercise intensity increases. The harder a person exercises, the higher their volume of oxygen utilized (VO2). People with higher values generally have greater energy reserves, increased work capacity, and are less-easily fatigued.

<i>Fitness Level (aerobic capacity)</i>	<i>VO₂ (ml/kg/min)</i>
	<i>20-29 years, Females</i>
Low (poor)	<24
Low-moderate (below average)	24-31
Moderate (average)	31-38
High-moderate (above average)	38-49
Very High (excellent)	>49



Blood Lactate: Lactic acid is produced in many of the cells of the body, particularly in muscle cells during exercise. From these cells lactic acid diffuses out into the blood where it can be measured (this is why we pricked your fingers and/or ears!). Accumulation of lactic acid occurs when the supply of oxygen to the cells is limited because the muscle cells are working so hard that the oxygen supply cannot keep up with the oxygen demand. During everyday activities and at low levels of exertion, the oxygen supply to the muscle cells is sufficient, and cells are able to utilize energy from various sources; this is known as the *aerobic (oxygen)* phase of exercise. When there is a sudden need for excessive muscular effort, the lactic acid mechanism is available to enable activity to continue even though the oxygen supply is insufficient. This is known as the *anaerobic (without oxygen)* phase of exercise.

During very high-intensity exercise, lactic acid will be produced faster than it can be removed, and it starts to accumulate in the working muscles, greatly reducing their efficiency. This causes an athlete discomfort (e.g. muscle burn), and ultimately results in reduced intensity or ending of the activity. As training progresses, the rate of lactate formation during exercise decreases, and the rate of lactate clearance (removal) increases during exercise. When exercise stops the lactate level falls much more slowly than during the build-up. This is the process of recovery from exercise, sometimes referred to as the warm-down or cool-down. Various factors influence the rate of recovery, such as an active cool-down period, which accelerates the rate of lactate fall.

From Endurance test:

% VO₂ max and lactate levels maintained during the 4 stages of the 4K Time Trial:

	<i>Baseline</i>		<i>Endpoint</i>	
			<i>%Change</i>	
	VO2	Lactate		
1000m	_____ %	_____ mmol/l	_____ %	_____ mmol/l
			_____ %	_____ %
2000m	_____ %	_____ mmol/l	_____ %	_____ mmol/l
			_____ %	_____ %
3000m	_____ %	_____ mmol/l	_____ %	_____ mmol/l
			_____ %	_____ %
4000m	_____ %	_____ mmol/l	_____ %	_____ mmol/l
			_____ %	_____ %
400 m time:	_____		_____	
			_____ %	

Treatment Group Assignment

For the eight-week study, you were assigned to receive the following:

Placebo (lactose filler)

Iron (1 capsule = 50 mg FeSO₄; 2 capsules/d = 100 mg FeSO₄)

Appendix 11 Daily training log

Daily Log Instructions

This training log should be completed on a **daily basis** for the next 7 days. Initial use of this log may take up to 5 minutes/day. For some questions, please rate each factor, as you feel **today** by placing a **solid vertical line** on the scale.

Example: Happy: How happy do you feel right now?

Not at all happy _____ | _____ Extremely happy

Physical Activities: record today's **physical activities**, sport-specific and general conditioning workouts, as well as **VIGOROUS** leisure-time physical activities lasting at least 10 minutes (examples: running to class, club sports, riding bike, swimming, playing active games with friends, etc).

Please record **total time** for each activity (Minutes). If applicable, record total distance (m, yards, mi, km); pace (mph, spm).

AM and PM workouts should be recorded separately.

Please include any **comments/thoughts** you have on your performance today (how well you felt, what you did or did not do well, etc).

Please rate activities' intensities in your log as follows:

1= I can talk easy, only light sweating, and/or a slight increase in breathing or heart rate (**low-Intensity**)

2= I can talk with some difficulty, light to moderate sweating, and/or a moderate increase in breathing or heart rate (**moderate Intensity**)

3= I can't talk comfortably, heavy sweating, and/or large increases in breathing or heart rate (**vigorous intensity**)

Please include the following information from each ergometer workout session:

Meters (distance) rowed on ergometer

Minutes (time) rowed on ergometer

Stroke rating(s) during piece(s) performed

Average split time (/500m) during piece(s) performed

(This section to be completed in the morning)

How many capsules did you consume today? _____ capsules.

If you did not consume any capsules today, please explain:

How long did you sleep last night? Length of sleep: _____ (hr).

Please rate the quality of last night's sleep:

Not at all restful _____ Extremely restful

How motivated are you to train today?

Not at all motivated _____ Extremely motivated

How do you feel today?

Not at all well _____ Extremely well

Please explain (note any GI, respiratory, severe headaches, etc):

Are you menstruating today? () Yes () No

(This section to be completed at the end of the day)

Did you have any ***sore joints &/or pains*** in your muscles today?

No pain/soreness at all _____ Severe pain/soreness

Please explain:

How ***fatigued*** did you feel today?

Not at all fatigued _____ Extremely fatigued

Please explain:

How much was your workout today affected by ***illness***?

Not at all affected _____ Could not work out today

Please explain:

Did you take a nap today? () Yes () No If Yes, how long? _____
minutes

Appendix 12. Exercise test data collection forms

VO₂peak

Best 2K erg split-time (maintained 30 sec) you can do right now: ____ min:sec = ____ W
(to be used for level 6)

Start test 5 levels above best split time:

Level	Split time	Watts
1		
2		
3		
4		
5		
6		
7		

Level	Stage-time	WR (W - monitor)	HR	Parvo time (min: sec)
1	0:30			
	1:00			
	1:30			
	0:10 Rest			
2	0:30			
	1:00			
	1:30			
	0:10 Rest			
3	0:30			
	1:00			
	1:30			
	0:10 Rest			
4	0:30			
	1:00			
	1:30			
	0:10 Rest			
5	0:30			
	1:00			
	1:30			
	0:10 Rest			
6	0:30			
	1:00			
	1:30			
	0:10 Rest			
7	0:30			
	1:00			
	1:30			
	0:10 Rest			

VO₂peak: ____ L/min at Level: ____ HRmax: ____ bpm WRmax: ____ W
WR at 85% VO₂peak: ____ L/min, ____ W ____ min

Endurance – 4K Time Trial(TT)

Interval	Parvo-time (min:sec)	WR (W)- monitor	HR
WR at 85% VO ₂ peak: _____ W. Start test at WR at 85% VO ₂ peak for the first 3600m (4- 1000m intervals). At last 400m (last 1000m level), increase WR (W) by pulling as hard or as fast as you can in order to sprint to finish.	0:30		
	1:00		
	1:30		
	2:00		
	2:30		
	3:00		
	3:30		
	4:00		
	4:30		
	5:00		
1000m	5:00		
Rest time start (1:00)			
Restart time			
	0:30		
	1:00		
	1:30		
	2:00		
	2:30		
	3:00		
	3:30		
	4:00		
	4:30		
2000m	5:00		
Rest time start (1:00)			
Restart time			
	0:30		
	1:00		
	1:30		
	2:00		
	2:30		
	3:00		
	3:30		
	4:00		
	4:30		
3000m	5:00		
Rest time start (1:00)			
Restart time			
	0:30		
	1:00		
	1:30		
	2:00		
	2:30		
	3:00		
	3:30		
Record Start sprint time (400m left)	4:00		
	4:30		
4000m	5:00		
Record total time to finish 4K			

VO₂peak ___ at ___ m, ___ bpm HR, ___ W, ___ spm; 85% VO₂peak ___ at ___ W