dietitianCE
Continuing Education for Nutrition Professionals

How Iron Status Is Related To Physical and Cognitive Health

Sex-specific relationships among iron status biomarkers, athletic performance, maturity, and dietary intakes in pre-adolescent and adolescent athletes

Abstract

Background: The purpose of this study was to examine relationships among biomarkers of iron status, athletic performance, growth and development, and dietary intakes in pre-adolescent and adolescent male and female athletes.

Methods: Two-hundred and forty-nine male ($n = 179$) (mean ± standard deviation for age = 12.0 ± 2.1 years, height = 156.3 ± 13.9 cm, and weight = 49.1 ± 16.5 kg) and female ($n = 70$) (12.0 ± 2.2 years, 152.4 ± 12.3 cm, 45.3 ± 14.5 kg) athletes volunteered for capillary blood sample, anthropometric, athletic performance, and dietary intake assessments. Outcomes included maturity offset from peak height velocity, percent body fat, estimated muscle cross-sectional areas, vertical jump height (VJ), broad jump distance (BJ), pro-agility time (PA), L-cone time, 20-yard dash time (20YD), power push up (PPU) force, dietary intakes, and ferritin, soluble transferrin receptor (sTfR), and hemoglobin (Hb) concentrations.

Results: Athletic performance was consistently correlated with Hb in males ($r = .237–.375$, $p < 0.001–0.05$) and with sTfR ($r = .521–.649$, $p < 0.001–0.004$) and iron intake ($r = .397–.568$, $p = 0.001–0.027$) in females. There were no relationships between dietary intakes and ferritin, sTfR, or Hb ($p > 0.05$). After partialing out age and height, VJ, PA, LC, and 20YD remained correlated with Hb in males ($|r_{Hb,y.Age}| = .208–.322$, $p = 0.001–0.041$; $|r_{Hb,y.Height}| = .211–.321$, $p = 0.001–0.038$). After partialing out iron intake, PA and LC remained correlated with sTfR in females ($|r_{sTfR,y.ironintake}| = .516–.569$, $p = 0.014–0.028$).

Conclusions: Iron status biomarkers demonstrated sex-specific relationships with anaerobic exercise performance in youth athletes, which may be more dependent on maturity status and dietary intake than age. Moderate relationships between sTfR and athletic performance in adolescent female athletes emphasizes the importance of iron intake in this demographic.

Keywords: Youth athletes, Exercise, Nutrition, Athletic performance, Iron

Background

Iron plays important roles for athletic performance, including red blood cell production, oxygen transport, and electron transport during oxidative phosphorylation [1–4]. Biomarkers used to measure iron status in athletes have included ferritin, soluble transferring receptor (sTfR), and hemoglobin (Hb) concentrations [5–7]. Previous studies have demonstrated positive associations between athletic performance measurements and ferritin and Hb concentrations [8–11], while sTfR concentrations have been inversely related to exercise [12]. Therefore, exercise and athletic performance is impacted by iron status, which suggests that maintaining adequate intakes of dietary iron may be important for athletes.

Dietary iron requirements for children are also important for healthy growth and development. Children have increased dietary iron requirements due to high growth rates of bone and muscle, increased plasma volumes, onset of menarche in females, and often inadequate consumption of dietary iron [5, 13, 14]. Given the increased popularity of competitive youth sports [15], youth athletes may exhibit a particularly high demand for dietary iron intake when considering both growth and development and athletic performance requirements.

An early study by Cullumbine [8] showed that speed and strength were related to Hb concentrations in adolescent males, but these relationships did not exist for females of the same age. The authors also reported greater performance scores and Hb concentrations in 14–20-year old males compared to females. Nearly 60 years later, Gracia-Marco and colleagues [10] reported remarkably similar relationships between Hb and both cardiorespiratory and muscular fitness in 12.5–17.5-year old males, but not females. Mechanisms exist for how oxygen transport and utilization can be related to anaerobic exercise performance, which may also provide a theoretical construct for relationships between Hb and anaerobic performance. For example, the use of aerobic metabolism is suggested to be predominant during adolescence, as measured levels of oxidative enzymes were higher in young males and females compared to adults [16, 17]. Since children rely more heavily on myoglobin-rich, oxidative fibers [17, 18], the oxygen carrying capacity of Hb or myoglobin may be more influential during anaerobic performance in children. Furthermore, the resynthesis of creatine phosphate within the mitochondria of skeletal muscle is oxygen-dependent [19, 20]. Given that 49–57% of children in the United States participate in team and individual sports [21] and nearly all those sports are anaerobic in nature, evaluating relationships among iron status and anaerobic performance in youth may appropriately reflect their state of health and physical activity.

While previous studies have demonstrated relationships between athletic performance and ferritin [9], sTfR [12], and Hb [11] in adult athletes, there is a lack of research directly relating concentrations reflecting iron status to athletic performance in young athletes. Overall, these previous studies [8–11] have raised questions about the relationships between Hb concentrations and aerobic versus anaerobic exercise performance as well as the potential value of relationships among ferritin and sTfR concentrations and exercise performance in females. However, results in adults cannot be extrapolated to pre-adolescent and adolescent athletes due to differences in energy utilization [22]. Previous studies examining young athletes reported prevalence of iron deficiency and anemia [5, 23], yet few studies [8, 24] examined direct relationships with athletic performance in this younger population.

Athletic differentiation between males and females is thought to occur during adolescence [25, 26]; thus, studying the role of iron in relation to athletic performance in adolescents by sex may also provide insight regarding dietary recommendations for optimizing their health. Therefore, the purpose of the present study was to examine the relationships among biomarkers of iron status, athletic performance, growth and development, and dietary intakes of young male and female athletes. It was hypothesized that while there would be a positive relationship among iron status and athletic performance based on previous studies, [8, 10, 11] sex differentiations pertaining to individual biomarkers would emerge based on differences in growth and development of young males and females.

Methods

Study design

A cross-sectional design was used to quantify relationships among athletic performance tests, measures of dietary intake, and hematological biomarkers of iron status in male and female adolescent athletes ages 5 to 18 years old.

Subjects

Male ($n = 179$) and female ($n = 70$) adolescent athletes ($n = 249$ total) volunteered for this study. Subjects were 5–18 years old and actively participating in school- or club-sponsored sports that held regular practices. Sports included baseball, basketball, cheerleading, cross country, dance, equestrian, football, golf, gymnastics, hockey, lacrosse, martial arts, rugby, soccer, softball, speed/power/agility training, swimming/diving, tennis, track and field, trap shooting, volleyball, weightlifting, and wrestling. Participants completed the Physical Activity Readiness Questionnaire for everyone (PAR-Q+ 2015), [27] that consists of general health questions to

determine if the participant is safe to engage in physical activity. This study was approved by the University of Nebraska-Lincoln Institutional Review Board for the protection of human subjects (IRB # 20160616246EP, Title: Youth Combine Testing, approval date: June 24, 2016). Each participant signed an approved youth assent form if they were 7–18 years old, and if the participant was 5–6 years old, verbal assent was obtained. One parent or legal guardian of each participant signed an approved informed consent document.

Anthropometrics and body composition

Height (cm) and weight (kg) were measured using a beam scale with attached stadiometer (Mechanical Column Scale & Stadiometer, Seca gmbh & co. kg, Hamburg, Germany). Seated height was measured to calculate maturity offset to predict peak height velocity (PHV) [28]. A maturity offset of less than − 0.5 years from PHV was considered pre-adolescent; − 0.5 to + 0.5 years from PHV was considered adolescent; and greater than + 0.5 years from PHV was considered post-adolescent [28, 29]. Body composition measurements included percent body fat (BF%), arm estimated cross-sectional area (eCSA), and thigh eCSA. Skinfold measurements were taken with a Lange caliper (Model 68,902, Cambridge Scientific Industries, Inc., Cambridge, MD, USA) and were used to calculate BF%. Skinfold measurements were taken on the right side of the body at the triceps (vertical fold in the middle of the upper arm, midway between the acromion and olecranon process) and anterior suprailiac (diagonal fold immediately superior to the anterior superior iliac spine) for males, and the triceps, suprailiac (diagonal fold 1 cm above the anterior superior iliac crest), and subscapula (diagonal fold 2 cm below the inferior angle of the scapula) for females. All skinfolds were recorded to the nearest 0.5 mm [30] and were entered into equations established by Housh et al. [31] and Brozek et al. [32] to estimated body density and BF%, respectively.

Arm and thigh circumferences were measured using a Gulick measurement tape (Baseline® measurement tape with Gulick attachment, Fabrication Enterprises, White Plains, NY) and recorded to the nearest 0.1 cm. Arm circumference and triceps skinfold were used to calculate arm eCSA, while thigh circumference and thigh skinfold (vertical pinch at the mid-point of the anterior surface of the thigh, halfway between the patella and inguinal fold) were used to calculate thigh eCSA using procedures described by Moritani and deVries [33].

Athletic performance testing

Detailed procedures of all athletic performance testing measurements are described by Gillen et al. [34]. Testing was conducted with similar methodology and equipment as the basic tests performed at the National Football League (NFL) scouting combine. Tests included the vertical jump (VJ), broad jump (BJ), pro-agility (PA), L-cone (LC), 20-yard dash (20YD) and power push up (PPU). The VJ was an assessment of vertical jumping performance measured with a Vertec (Sports Imports, Freestanding Vertec Jump Trainer, Hilliard, OH, USA) and was calculated as the difference between standing reach and the highest jump recorded (cm). BJ assessed horizontal jumping performance as the distance between the starting line and the heel of the subject closest to the starting line (cm). The two agility drills, PA and LC, and the 20YD, were measured in seconds (s) using a digital, laser beam actuated timing gate with motion start (Brower Timing Systems, Brower TC Motion Start Timer, Knoxville, TN, USA). Splits were recorded at 5 and 10 yards during the 20YD.

Dietary intake assessments

Among the total sample ($n = 249$), 39% ($n = 97$; male, $n = 66$; female, $n = 31$) also completed a 24-h dietary recall administered online using the Automated Self-Administered 24-h (ASA24®) Dietary Recall System. If the participant was less than 14 years old, the recall was administered to a parent or legal guardian for completion. Participants were prompted with detailed questions regarding food intake with regard to serving sizes and composition of food choices. Total energy ($kcal \cdot d^{-1}$), carbohydrate ($g \cdot d^{-1}$), protein ($g \cdot d^{-1}$), fat ($g \cdot d^{-1}$), and iron ($mg \cdot d^{-1}$) intakes were quantified and reported from the ASA24®.

Biomarkers of Iron status

Capillary blood samples of 400 μL were collected in microvettes (Microvette® 200 μL, K3 EDTA, violet US code; 10.8 mm × 46.6 mm) to analyze ferritin and sTfR. Human alpha 1-acid glycoprotein (AGP) was assessed to determine inflammatory status of the participant to correct ferritin concentrations if falsely elevated [35]. Enzyme-linked immunosorbent assay (ELISA) kits were used to assess concentrations of ferritin ($\mu g \cdot L^{-1}$; $n = 118$; males, $n = 94$; females, $n = 24$) (ELISA kit Ramco Labs), sTfR ($nmol \cdot L^{-1}$; $n = 105$; males, $n = 76$; females, $n = 29$) (Quantikine IVD ELISA Kit, R&D Systems), and AGP ($\mu mol \cdot L^{-1}$; $n = 40$; males, $n = 39$; females, n = 1) (ELISA kit, R&D Systems). AGP was quantified in a lower sample, since after the first 40 assays, none exhibited a high enough inflammatory status to warrant a correction of ferritin. Assay procedures were followed per kit instructions and absorbance was read at 500 and 650 nm for ferritin and 450 and 540 nm for sTfR and AGP. Hemoglobin (Hb) concentration ($g \cdot L^{-1}$) was assessed on site during the athletic performance testing with a handheld hemoanalyzer (AimStrip®*Hb* Hemoglobin meter, Germaine Laboratories, Inc.) in 51% of the total sample ($n = 128$; male, $n = 100$; female, $n = 28$).

Statistical analyses

Means and standard deviations for anthropometrics, per-formance measurements, dietary intakes, and biomarkers of iron status were calculated in a spreadsheet software program (Microsoft Excel 2017, version 16.10) (Table 1). Exploratory data analysis for outliers was performed using the Tukey procedure [36]. Independent-samples t-tests (with unequal variances assumed) were used to compare the mean values of males versus females (Table 1). A Pear-son product moment correlation analysis was performed with and without outliers, among all 7 descriptive and anthropometric variables, 6 performance variables, 5 dietary intake variables, and 3 biomarkers of iron status for all athletes (Table 2) and separated by males and females. Correlation coefficients were evaluated qualitatively according to Mukaka [37]: 0.00 to 0.30 = negligible; 0.30 to 0.50 = low; 0.50 to 0.70 = moderate; 0.70 to 0.90 = high; 0.90 to 1.00 = very high. For significant collinear relationships among anthropometrics, athletic performance, dietary intakes, and iron status biomarkers, first-order partial correlations (r_{xyz}) were calculated to partial out collinear influences. All statistical analyses were performed using IBM SPSS Statistics for Macintosh, Version 24 (IBM Corp., Chicago, IL, USA.) An alpha of $p \leq 0.05$ was considered statistically significant for all correlations and comparisons.

Results

Outliers ($n = 16$) were identified for weight ($n = 1$), BF% ($n = 1$), arm eCSA ($n = 1$), PA ($n = 1$), LC ($n = 1$), 20YD ($n = 2$), PPU ($n = 2$), iron ($n = 4$), and sTfR ($n = 3$), and the values for each outlier are reported in Table 1. The independent samples t-tests showed significant differences between males and females for maturity offset, height, arm eCSA, thigh eCSA, VJ, BJ, PA, LC, PPU, ferritin, energy intake, protein, carbohydrates, and iron ($p < 0.001–0.048$). With outliers removed, the sex difference in 20YD time became significant ($p = 0.041$), while the sex difference in iron intake became non-significant ($p = 0.104$) (Table 1).

Table 2 illustrates the significant interrelationships ($p \leq 0.05$) among the anthropometric measurements in

Table 1 Demographics, anthropometrics, athletic performance scores, dietary intakes and biomarkers of iron status

	Composite ($n = 249$)	Males ($n = 179$)	Females ($n = 70$)	Outliers ($n = 16$)
Age (y)	12.0 ± 2.1 ($n = 249$)	12.0 ± 2.1 ($n = 179$)	12.0 ± 2.2 ($n = 70$)	
Maturity Offset (y)	−1.3 ± 1.9 ($n = 249$)	−1.7 ± 1.7 ($n = 179$)*	−0.1 ± 1.8 ($n = 70$)	
Height (cm)	155.2 ± 13.6 ($n = 249$)	156.3 ± 13.9 ($n = 179$)*	152.4 ± 12.3 ($n = 70$)	
Weight (kg)	48.0 ± 16.0 ($n = 249$)	49.1 ± 16.5 ($n = 179$)	45.3 ± 14.5 ($n = 70$)	120.6 kg
Body Fat (%)	20.2 ± 6.5 ($n = 244$)	19.7 ± 6.7 ($n = 175$)	21.5 ± 5.9 ($n = 69$)	47.9%
Arm eCSA (cm^2)	14.3 ± 6.9 ($n = 246$)	15.6 ± 7.3 ($n = 176$)*	11.2 ± 4.6 ($n = 70$)	45.74 cm^2
Thigh eCSA (cm^2)	80.6 ± 31.5 ($n = 245$)	83.3 ± 33.0 ($n = 175$)*	73.7 ± 26.3 ($n = 70$)	
Vertical Jump (cm)	40.2 ± 9.4 ($n = 246$)	41.7 ± 9.6 ($n = 177$)*	36.4 ± 7.4 ($n = 69$)	
Broad Jump (cm)	168.6 ± 30.2 ($n = 247$)	172.9 ± 30.6 ($n = 178$)*	157.3 ± 26.2 ($n = 69$)	
Pro-Agility (s)	5.8 ± 0.6 ($n = 247$)	5.7 ± 0.6 ($n = 177$)*	5.9 ± 0.5 ($n = 70$)	8.76 s
L Cone (s)	9.4 ± 0.9 ($n = 245$)	9.3 ± 1.0 ($n = 176$)*	9.7 ± 0.8 ($n = 69$)	15.0 s
20 Yard Dash (s)	3.7 ± 0.5 ($n = 248$)	3.7 ± 0.5 ($n = 178$)[a]	3.8 ± 0.4 ($n = 70$)	5.98, 6.79 s
Power Push Up (N)	170.6 ± 84.1 ($n = 246$)	185.5 ± 90.0 ($n = 177$)*	132.6 ± 51.3 ($n = 69$)	583, 601 N
Energy Intake ($kcals{\cdot}d^{-1}$)	2052 ± 711 ($n = 97$)	2158 ± 749 ($n = 66$)*	1827 ± 568 ($n = 31$)	
Carbohydrates ($g{\cdot}d^{-1}$)	244 ± 89 ($n = 97$)	256 ± 89 ($n = 66$)*	217 ± 83 ($n = 31$)	
Protein ($g{\cdot}d^{-1}$)	90 ± 38 ($n = 97$)	98 ± 41 ($n = 66$)*	74 ± 25 ($n = 31$)	
Fat ($g{\cdot}d^{-1}$)	82 ± 37 ($n = 97$)	84 ± 39 ($n = 66$)	76 ± 34 ($n = 31$)	
Iron ($mg{\cdot}d^{-1}$)	16.5 ± 9.7 ($n = 97$)	17.9 ± 10.9 ($n = 66$)*[b]	13.5 ± 5.5 ($n = 31$)	46.0, 44.8, 55.0, 62.0 $mg{\cdot}d^{-1}$
Hemoglobin ($g{\cdot}L^{-1}$)	113 ± 16 ($n = 128$)	114 ± 16 ($n = 100$)	112 ± 19 ($n = 28$)	
Ferritin ($\mu g{\cdot}L^{-1}$)	24.0 ± 15.0 ($n = 118$)	25.3 ± 16.2 ($n = 94$)*	18.6 ± 7.3 ($n = 24$)	
sTfR ($nmol{\cdot}L^{-1}$)	22.1 ± 6.4 ($n = 105$)	21.9 ± 6.8 ($n = 76$)	22.8 ± 5.5 ($n = 29$)	38.8, 44.5, 66.7 $nmol{\cdot}L^{-1}$

Values are means ± standard deviations (SD)
*Indicates a significant difference between the mean values of males versus females ($p \leq 0.05$) with outliers included
[a]Indicates a significant difference after removal of outliers. [b]Indicates difference became non-significant after removal of outliers

Table 2 Pearson product moment correlation coefficient matrix among all variables for composite sample of young athletes

	Age		Maturity Offset		Height		Weight		BF%		Arm eCSA		Thigh eCSA		VJ		BJ		PA		LC	
N pairs	249		249		249		249		244		246		245		246		247		247		245	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N pairs	179	70	179	70	179	70	179	70	175	69	176	70	175	70	177	69	178	69	177	70	176	69
Maturity Offset	.875[a]																					
	.946[a]	.972[a]																				
Height	.837[a]		.802[a]																			
	.839[a]	.859[a]	.927[a]	.945[a]																		
Weight	.690[a]		.714[a]		.800[a]																	
	.669[•]	.682[a]	.841[a]	.779[a]	.812[a]	.748[a]																
BF%	−.103		−.034		−.056		.352[a]															
	−.260[a]	.333[a]	−.173[ac]	.425[a]	−.166[a]	.378[a]	.250[a]	.775[a]														
Arm eCSA	.459[a]		.347[a]		.506[a]		.463[a]		−.388[a]													
	.507[a]	.386[a]	.550[a]	.430[a]	.519[a]	.398[a]	.416[a]	.639[a]	−.508[a]	.251[ac]												
Thigh eCSA	.666[a]		.632[a]		.704[a]		.716[a]		−.073		.591[a]											
	.667[a]	.696[a]	.754[a]	.747[a]	.692[a]	.726[a]	.721[a]	.681[a]	−.140[a]	.245[ac]	.573[a]	.638[a]										
VJ	.636[a]		.447[a]		.586[a]		.321[a]		−.496[a]		.566[a]		.537[a]									
	.674[a]	.616[a]	.627[a]	.613[a]	.575[a]	.593[a]	.303[a]	.321[a]	−.585[a]	−.097	.569[a]	.336[a]	.509[a]	.593[a]								
BJ	.639[a]		.452[a]		.571[a]		.290[a]		−.453[a]		.530[a]		.526[a]		.844[a]							
	.656[a]	.664[a]	.589[a]	.669[a]	.527[a]	.683[a]	.261[a]	.323[a]	−.544[a]	.083	.558[a]	.225	.499[a]	.564[a]	.878[a]	.663[a]						
PA	−.635[a]		−.434[a]		−.512[a]		−.184[a]		.496[a]		−.401[a]		−.382[a]		−.820[a]		−.803[a]					
	−.621[a]	−.716[a]	−.523[a]	−.673[a]	−.465[a]	−.619[a]	−.144[b]	−.249[a]	.608[a]	.069	−.423[a]	−.127	−.323[a]	−.509[a]	−.840[a]	−.713[a]	−.834[a]	−.662[a]				
LC	−.606[a]		−.410[a]		−.493[a]		−.201[a]		.473[a]		−.433[a]		−.369[a]		−.762[a]		−.748[a]		.920[a]			
	−.600[a]	−.654[a]	−.505[a]	−.610[a]	−.462[a]	−.545[a]	−.165[a]	−.251[a]	.581[a]	.055	−.462[a]	−.134	−.314[a]	.479[a]	−.761[a]	−.726[a]	−.773[a]	−.612[a]	.926[a]	.885[a]		
20YD	−.593[a]		−.428[a]		−.485[a]		−.151[a]		.470[a]		−.349[a]		−.345[a]		−.754[a]		−.734[a]		.874[a]		.811[a]	
	−.569[a]	−.661[a]	−.476[a]	−.619[a]	−.399[a]	−.595[a]	−.100[b]	−.255[ac]	.600[a]	.066	−.386[a]	−.129	−.289[a]	−.471[a]	−.767[a]	−.721[a]	.777[a]	−.600[a]	.885[a]	.847[a]	.843[a]	.709[a]
PPU	.585[a]		.478[a]		.598[a]		.660[a]		−.041		.508[a]		.664[a]		.485[a]		.520[a]		−.399[a]		−.396[a]	
	.680[a]	.381[a]	.754[a]	.390[a]	.650[a]	.343[a]	.731[•]	.350[a]	−.039	.158	.497[a]	.239[a]	.719[a]	.343[a]	.491[a]	.190	.482[a]	.423[a]	−.397[a]	−.232	−.393[a]	−.216
Hb	.184[a]		.139		.194[a]		.159		−.037		.075		.086		.247[a]		.214[a]		−.230[a]		−.228[a]	
	.202[a]	.145	.188	.157	.219[a]	.101	.112	.283	−.147	.288	.031	.205	.102	.026	.331[a]	−.002	.277[a]	.037	−.317[a]	.064	−.284[a]	−.034
Ferritin	−.016		−.027		.099		.252[a]		.278[a]		−.049		−.052		−.137		−.166		.181		.141	
	.019	−.011	.045	.081	.063	.195	.246[a]	.345	.323[a]	.257	−.137	.386	−.087	.130	−.201	−.184	−.272[a]	.150	.222[a]	.360	.188	.313
sTfR	.009[b]		.099		.050		.061		.005		.048		.046		−.093[b]		−.047		.073[b]		.088[b]	
	.126	−.295	.196	−.240	.132	−.182	.093	−.001	−.067[b]	.200	.125	−.183	.132	−.215	.084	−.562[a]	.068	−.308	−.079	.522[a]	−.092	.649[a]

Table 2 Pearson product moment correlation coefficient matrix among all variables for composite sample of young athletes *(Continued)*

	Age		Maturity Offset		Height		Weight		BF%		Arm eCSA		Thigh eCSA		VJ		BJ		PA		LC	
N pairs	249		249		249		249		244		246		245		246		247		247		245	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Energy Intake	.047		−.054		.088		−.052		−.298[a]		.182		.112		.276[a]		.253[a]		−.266[a]		−.252[a]	
	.051	.015	.067	−.045	.084	−.041	−.068	−.250	−.281[a]	−.344	.162	−.076	.139	−.084	.263[a]	.188	.187	.240	−.265[a]	−.102	−.186	−.233
Protein	.153		.041		.247[a]		.195		−.162		.325[a]		.288[a]		.312[a]		.260[a]		−.237		−.217[a]	
	.251[a]	−.132	.285[a]	−.141	.280[b]	−.46	.202	−.169	−.138	−.232	.295[ac]	.020	.352[a]	−.096	.290[a]	.213	.201	.139	−.213	−.054	−.144	−.142
Fat	.045		.003		.076		−.039		−.210[a]		.071		.122		.174		.154		−.179		−.140	
	.035	.056	.061	.002	.080	.002	−.038	−.162	−.198	−.231	.055	−.033	.179	−.103	.164	.148	.112	.174	−.173	−.121	−.093	−.150
Carbo-hydrate	−.029		−.132		−.011		−.170		−.340[a]		.144		.024		.245[a]		.243[a]		−.253[a]		−.271[a]	
	−.066	.019	−.071	−.031	.043	−.051	−.222	−.229	−.342[a]	−.324	.139	−.129	−.056	−.024	.249[a]	.120	.180	.222	−.277[a]	−.054	−.219	−.231
Iron	.138		.032		.153		−.029		−.274[a]		.091[b]		.162		.312[a]		.215[ac]		−.220[ac]		−.224[ac]	
	.061	.436[a]	.069	.387[a]	.086	.323	−.109	.162	−.264[ac]	−.314	−.011	.363[a]	.072	.535[a]	.225	.565[a]	.078	.568[a]	−.130	−.397[a]	−.177	−.465[a]

The top row indicates the correlation with all athletes and the bottom row indicates the correlation separated by male (M) on the left side and female (F) on the right side

[a]Correlation is significant at the 0.05 level with outliers included; [b]Correlation became significant with removal of outliers; [c]Correlation became non-significant with removal of outliers

Table 2 Pearson product moment correlation coefficient matrix among all variables for composite sample of young athletes *(Continued)*

	20YD		PPU		Hb		Ferritin		sTfR		Energy Intake		Protein	Fat		Carbohydrate		
N pairs	248		246		128		118		105		97		97	97		97		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N pairs	178	70	177	69	100	28	94	24	76	29	66	31	66	31	66	31	66	31
Maturity Offset																		
Height																		
Weight																		
BF%																		
Arm eCSA																		
Thigh eCSA																		
VJ																		
BJ																		
PA																		
LC																		
20YD																		
PPU	−.346[a]																	
	−.373[a]	−.157																
Hb	−.204[a]		.221[a]															
	−.375[a]	.177	.237[a]	.146														
Ferritin	.181[a]		.070		.065													
	.223[a]	.200	.033	−.034	.000	.606[a]												
sTfR	.070[b]		−.011		.033		−.223[ac]											
	−.132	.521[a]	−.001	.126	.023	.086	−.262[ac]	.228										

Table 2 Pearson product moment correlation coefficient matrix among all variables for composite sample of young athletes *(Continued)*

	20YD		PPU		Hb		Ferritin		sTfR		Energy Intake		Protein	Fat		Carbohydrate		
N pairs	248		246		128		118		105		97		97	97		97		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Energy Intake	−.205[a]		.088		−.080		−.053		−.272[a]									
	−.197	−.146	−.101	−.326	−.133	.040	−.101	.236	−.297	−.278								
Protein	−.193[b]		.208[ac]		.017		.131		−.222		.763[a]							
	−.162	−.151	.215	−.326	−.030	.143	.093	.244	−.234	−.198	.789[a]	.577[a]						
Fat	−.133		.096		−.194		−.117		−.218		.895[a]		.700[a]					
	−.105	−.143	.130	−.186	−.185	−.238	−.093	−.151	−.195	−.312	.916[a]	.847[a]	.731[a]	.623[a]				
Carbo-hydrate	−.199[b]		−.018		.010		−.052		−.248[b]		.839[a]		.425[a]		.551[a]			
	−.229	−.080	−.032	−.288	−.094	.237	−.162	.454	−.341[a]	−.125	.852[a]	.781[a]	.469[a]	.114	.614[a]	.357[a]		
Iron	−.242[ac]		.001		.074		.112		−.240		.414[a]		.348[a]		.271[a]		.440[a]	
	−.155	−.466[a]	−.056	−.130	.030	.223	.055	.365	−.217	−.341	.392[a]	.377[a]	.329[a]	.155	.288[a]	.126	.414[a]	.476[a]

the composite sample and separated by sex. Specifically, age, maturity offset, height, weight, and thigh eCSA demonstrated moderate to very high intercorrelations. Arm eCSA showed low intercorrelations among females and moderate intercorrelations among males. Therefore, age, maturity offset, height, weight, and thigh eCSA were interpreted to collectively reflect growth and development in females, while arm eCSA was added to the same group of variables to reflect growth and development in males. BF% showed mostly negligible to low intercorrelations and was subsequently excluded from growth and development (Table 2).

Similarly, the VJ, BJ, PA, LC, and 20YD measurements were consistently interrelated at a significant level ($p \leq 0.05$) within the composite sample as well as the separate male and female correlation matrices. The direction of the correlation reflected the measurement (distance, time, or power) such that better performance occurred with greater distance (VJ or BJ) and greater power (PPU), whereas better performance occurred with lower time-scored variables (PA, LC, and 20YD). Intercorrelations among VJ, BJ, PA, LC, and 20YD were all high or very high, except for BJ in the females, which exhibited moderate intercorrelations. Therefore, these variables were interpreted to collectively reflect athletic performance (Table 2). PPU scores exhibited negligible to low intercorrelations among the other vari-ables and was subsequently excluded from the grouping.

From the ASA24®, energy, carbohydrate, protein, fat, and iron intakes demonstrated consistent, but not uni-form, significant intercorrelations ($p \leq 0.05$). Iron exhib-ited mostly negligible to low relationships among the other dietary intakes. By virtue of how these variables were collected and reported, all were collectively inter-preted as dietary intakes; however, they were also con-sidered individually for relationships with growth and development, athletic performance, and biomarkers of iron status (Table 2).

The biomarkers for iron status (ferritin, sTfR, and Hb) were not consistently intercorrelated (Table 2). The relationship between ferritin and Hb was significant ($p \leq 0.05$) and moderate in magnitude in females only, and the relationship between ferritin and sTfR in the composite sample and in males became non-significant ($p \geq 0.05$) after the removal of outliers. However, the magni-tudes of the intercorrelations among ferritin, sTfR, and Hb were mostly negligible. Therefore, each bio-marker was examined separately.

Correlations among growth and development, athletic performance, dietary intake, ferritin, sTfR, and Hb are also illustrated in Table 2 and Fig. 1. Overall, growth and development was significantly ($p < 0.001–0.048$) correlated with athletic performance with magnitudes ranging from low to moderate. Age, maturity offset, and height exhibited nearly uniform, moderate correlations with athletic performance. Arm and thigh eCSA values were moderately correlated with VJ and BJ performances in males, while only thigh eCSA was moderately related to VJ and BJ performances in females. In males only, BF% exhibited moderate, inverse relationships with athletic performance, and PPU was moderately related to growth and development. Other significant ($p \leq 0.05$) relationships among growth and development and athletic performance were low in magnitude.

Growth and development variables were not consistently related to dietary intakes, ferritin, sTfR, or Hb, with two exceptions. First, growth and development exhibited negligible, but significant ($p = 0.004–0.042$), relationships with protein intake in males. Second, growth and development displayed low to moderate relationships ($p = 0.002–0.045$) with iron intake in females.

Athletic performance was not consistently related to ferritin, sTfR, or Hb in the composite sample. However, when separated by sex, athletic performance exhibited consistent, negligible to low correlations with Hb in males ($p < 0.001–0.05$). Athletic performance also displayed consistent, low to moderate correlations with sTfR in females ($p < 0.001–0.004$). Figure 1 illustrates the relationships among athletic performance and Hb in the males (left scatterplots) as well as predominantly moderate correlations among athletic performance and sTfR in the females (right scatterplots).

Athletic performance exhibited consistent, negligible ($p \leq 0.05$) relationships with energy and macronutrient intakes in the composite sample. When separated by sex, VJ and PA still displayed negligible relationships with energy and carbohydrate intake in males ($p = 0.024–0.045$). In females, athletic performance exhibited consistent, moderate correlations with iron intake ($p = 0.001–0.027$). Dietary intakes were unrelated to ferritin, sTfR, or Hb with mostly negligible correlations.

When focusing on the relationships among athletic performance, dietary intakes, and ferritin, sTfR, and Hb, partial correlations were calculated to remove the influence of concurrently related (possibly collinear) growth and development or dietary intake variables. In males, the partial correlations for Hb and athletic performance, while partialing out age and height were still significant for four of the six athletic performance tests: VJ, PA, LC, and 20YD ($|r_{Hb,y.Age}| = .208–.322$, $p = 0.001–0.041$ and $|r_{Hb,y.Height}| = .211–.321$, $p = 0.001–0.038$), respectively. After partialing out weight from the correlations between ferritin and three athletic performance tests (BJ, PA, and 20YD), the relationships were still significant ($|r_{Ferritin,y.Weight}| = .257–.360$, $p < 0.001–0.013$). However, after partialing out BF%, the relationships between ferritin and athletic performance disappeared ($|r_{Ferritin,y.BF\%}| = .035–.122$, $p > 0.05$). Partial correlations in males for athletic

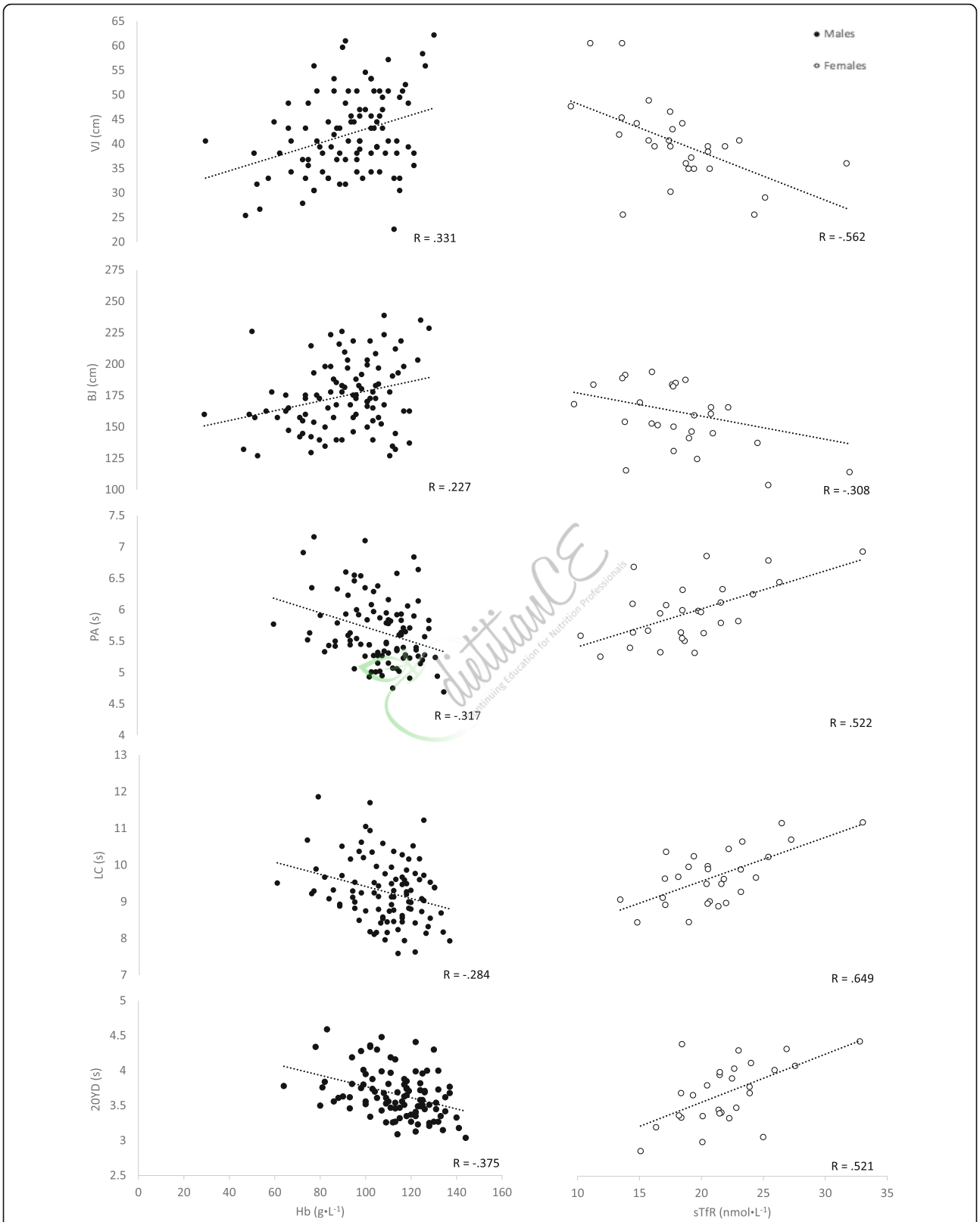

Fig. 1 Scatterplots showing the sex separation of the relationships between athletic performance and biomarkers of iron status. Athletic performance was correlated with Hb in males (closed circles), while athletic performance was related to sTfR in females (open circles), both in the direction that was expected

performance and Hb after partialing out dietary intakes were also still significant ($|r_{Hb,y.energyintake}|$ = .369–.383, p = 0.005–0.007 and ($|r_{Hb,y.carbohydrate}|$ = .249–.368, p = 0.007–0.009). For females, the partial correlations in two athletic performance tests (PA and LC) with sTfR remained significant after partialing out iron intake ($|r_{sTfR,y.ironintake}|$ = .516–.569, p = 0.014–0.028), but the relationship between sTfR and the other performance tests (VJ and 20YD) were no longer significant ($|r_{sTfR,y.ironintake}|$ = .028–.460, $p > 0.05$).

Discussion

The primary findings of the present study indicated that athletic performance was moderately related to sTfR concentrations in the female adolescent athletes, while athletic performance exhibited low correlations with Hb concentrations in the male pre-adolescent athletes (Fig. 1). Athletic performance was also moderately related to dietary iron intake in the females. Yet, conversely, there were no consistent relationships among dietary intakes, ferritin, sTfR, or Hb concentrations. Thus, dietary intake data was unable to track the biomarkers of iron status, but athletic performance, particularly in the female adolescent athletes, was directly proportional to sTfR and dietary iron intake. Although these correlations cannot infer causal relationships between sTfR or dietary iron intake and athletic performance, these findings can be hypothesis-generating.

Previous studies have established links between exercise performance and iron status in adult female athletes [9, 11, 12]. For example, active young adult females exhibited higher sTfR concentrations than sedentary females, but no other iron biomarkers were different between the two groups [12]. In female collegiate athletes, a positive relationship was reported between ferritin and VO_{2peak} [9]. The authors reported that a slower 4-km time trial performance was associated with iron depletion, but again no other relationships were observed with other iron status biomarkers [9]. Improvements in skeletal muscle strength were related to changes in Hb concentration following dietary iron supplementation in adult female elite volleyball players [11], but no other iron biomarker was related.

Interestingly, the adolescent female athletes in the present study demonstrated an inverse relationship between sTfR concentrations and athletic performance. That is, measures of athletic performance improved as sTfR concentrations decreased in the females (Table 2). Since sTfR is inversely proportional to iron availability [38, 39], which is thought to reflect erythropoiesis [39], red blood cell availability and function during athletic performance testing may be affected. It is possible that the iron availability in adolescent female athletes during their PHV may not be capable of supporting the demand for red blood cell production, thereby hindering skeletal muscle performance. Our findings may also tentatively suggest that the sTfR biomarker may be more sensitive than the other iron biomarkers in adolescent female athletes experiencing rapid growth, compared to adult athletic females.

Concentrations of Hb have also been associated with exercise and performance in young males [8, 10]. Cullumbine [8] reported low correlations between Hb and 100-yard sprint time (r = – 0.360) and deadlift strength (r = 0.440) in 14–20-year-old males. Gracia-Marco et al. [10] reported negligible, but significant, associations between Hb and BJ performance in 12.5–17.5-year-old males before (β = 0.286, $p < 0.001$) and after (β = 0.203, p = 0.001) covarying for age, seasonality, latitude, BMI, and moderate-to-vigorous physical activity level. The results of the present study demonstrated similar negligible to low correlations between Hb and VJ, BJ, PA, LC, 20YD and PPU in the pre-adolescent males (Fig. 1). The previous studies [8, 10] included older males (average age of 15 years) and reported higher average Hb concentrations (147 ± 12 and 151 ± 2 $g{\cdot}L^{-1}$, respectively) than the present study. Furthermore, neither previous study measured or accounted for biological maturity or muscle mass. The uniqueness of the present study included younger males (Table 1), lower Hb concentrations (Table 1), no relationships between Hb and maturity offset or Hb and muscle mass (Table 2), and the partial correlations that removed the influences of age and height from the correlations between Hb and athletic performance. Our findings suggested that even after removing the influence of growth and development, the relationships between Hb and athletic performance were still significant in these pre-adolescent male athletes.

The presence of an association between Hb concentration and strength, speed, or power measurements suggests that Hb may influence anaerobic exercise performance. Given the oxygen-carrying capacity of Hb, relationships between Hb and aerobic fitness are expected and have been demonstrated in adults [40–42]. Since anaerobic exercise performance is theoretically independent of oxygen availability, relationships between Hb and anaerobic performance are more difficult to explain. Interestingly, all the athletic performance measures in the present study are anaerobic in nature, and many previous studies have demonstrated associations between anaerobic exercise performance and iron status [8, 10, 11, 43]. For example, the strength of association between Hb and BJ reported by Gracia-Marco et al. [10] was greater than the strength of association between Hb and cardiorespiratory fitness in the same sample (β = 0.192, p = 0.002). Potential physiological explanations may

include the predominant, but not exclusive, anaerobic metabolism utilized, particular in children who rely more on oxidative mechanisms [16, 17, 44] and/or the oxygen-dependent resynthesis of creatine phosphate in the mitochondria [19, 20]. These relationships in children may also be impacted by a higher reliance on myoglobin-rich, oxidative fibers [18], allowing the oxygen carrying capacity of Hb to be more influential during anaerobic power, agility, and speed. Future studies are needed to test the hypotheses generated by the present and previous [8, 10] cross-sectional, correlational studies.

In an early study, Cullumbine [8] stated that "… males are faster than females and they have a greater strength at all ages; they also have consistently higher blood hemoglobin levels" (p. 276). Yet, the results of the present study did not entirely support the findings of Cullumbine [8]. In contrast to Cullumbine [8], there were no differences between the males and females in Hb or sTfR concentrations. When considering all measured variables, the largest sex differences were 32 to 40% greater upper-body strength (PPU) and muscle mass (arm eCSA), protein and iron dietary intakes, and ferritin concentrations. Moderate sex differences (10 to 18%) were evident in lower-body power (BJ and VJ), lower-body muscle mass (thigh eCSA), and energy and carbohydrate intakes. All other variables, including sprint speed (20YD), agility (PA and LC), fat intake, and Hb and sTfR concentrations were either equivalent or < 5% different between these young male and female athletes. Differences in upper-body, and to a lesser extent lower-body, strength and muscle mass are well-documented between boys and girls of this age [25, 26, 45]. Less is known about the dietary intakes and iron status biomarkers in relation to performance among this demographic. Since dietary intakes are reasonably modifiable, we would recommend increasing protein and iron intakes in young female athletes of this age. Future studies are needed to examine whether following such dietary recommendations results in improved ferritin concentrations and possibly athletic performance outcomes.

Despite the similarity in chronological age between the males and females in the present study, the females were experiencing a growth spurt (− 0.5 to + 0.5 years of maturity offset) at the time of data collection. In contrast, the males were 1.7 years away from their growth spurt (Table 1). This discrepancy between chronological age and biological maturity highlights the importance of interpretations involving growth and development. Previous research has hypothesized differences between young males and females in the timing of athletic devel-opment [25, 26], dietary needs and biomarkers of iron status [46]. The results of the present study extended existing knowledge by reporting relationships between growth and development and dietary iron intake in the adolescent female athletes, which was not observed in the pre-adolescent males (Table 2). Rossander-Hulthen and Hallberg [47] reported that starting at age 12, total estimated iron requirements increase in adolescent females, coinciding with the onset of menses. Adolescent females may need as much as 2.1 $mg \cdot d^{-1}$ of dietary iron intake [47]. For comparison in adolescent males during their PHV, dietary iron requirements for the 50th percentile is approximately 1.8 $mg \cdot d^{-1}$ [47]. However, the pre-adolescent males in the present study had not yet reached their growth spurt, which may explain why their dietary iron intake was not as related to growth and development as the females.

In contrast to dietary iron intake, dietary protein intake was related to growth and development in the males, but not the females in the present study (Table 2). Our findings supported those of previous studies [48, 49] related to protein intake and growth and development in young, growing males and females. Aerenhouts et al. [48] reported that on average, fat-free mass increased 2.44 $kg \cdot year^{-1}$ and 3.84 $kg \cdot year^{-1}$ in females and males, respectively, corresponding to protein accrual of 1.30 $g \cdot d^{-1}$ in females and 2.04 $g \cdot d^{-1}$ in males. These previous findings [48] suggest that the higher rate of skeletal muscle growth generally experienced in males may be associated with greater dietary protein needs for the younger, pre-adolescent males in the present study. Spear et al. [49] also suggested that protein needs of adolescents relate better to growth patterns than chronological age, especially in relation to height and tissue growth. Future studies may be needed to examine the relationships among growth and development measures and dietary protein intakes in males and females matched for biological maturity, rather than chronological age as is the case in the present study.

To further examine the relationships between athletic performance and Hb in males and sTfR in females, partial correlations were performed to see whether the relationships diminished after removing the influences of growth and development or dietary intakes. Neither growth and development (age and height) nor dietary intake (energy and carbohydrates) impacted the observed relationships between Hb and athletic performance. These findings suggest that Hb concentration is related to vertical power (VJ), agility (PA and LC), and speed (20YD) measures in pre-adolescent males, independent of growth and development or dietary intake. These findings, in conjunction with previous studies demonstrating relationships between Hb and anaerobic performance [8, 10, 11, 43], suggested that the oxygen-carrying role of Hb is at least partially related to anaerobic exercise performance. Since pre-adolescent children (only the males in the present study) tend to display type I muscle fiber characteristics [50], and type I fibers are heavily dependent on myoglobin [51], the associations between Hb and anaerobic exercise may be maturity-

dependent. However, this hypothesis does not explain similar relationships observed between Hb and anaerobic performance in adults [11].

In addition, removing the influence of iron intake eliminated the relationships between sTfR concentrations and VJ and 20YD performance in the females. Therefore, iron intake at least partially explained the relationships between sTfR concentrations and athletic performance. This finding tentatively suggests that improving dietary iron intake could potentially improve athletic performance in adolescent females, particularly with regard to VJ and 20YD performance. Future studies are needed, however, to experimentally verify this hypothesis. The overall contrasting differences between the effects of partialling out collinear variables between males and females in the present study may have reflected differences in biological maturity, emphasizing the importance of maturity, rather than age, when monitoring diet and athletic performance in young athletes.

One limitation of the study is the initial recruitment of participants by age instead of maturity status. The study was designed to be field-test friendly to allow many young athletes to participate. The participants were recruited across the age range of 5–18 years old in order to be able to assess males and females falling into cat-egories of pre-adolescent, adolescent, and post-adolescent. While categorizing by maturity status would be ideal due to the influence maturation has on iron require-ments, hemoglobin levels, and athletic performance, this was not feasible for this particular study due to the recruitment and testing strategies utilized.

A potential limitation to this study was that only 39% of the total sample completed the online dietary recall. However, the correlations and partial correla-tions involving dietary intakes were performed with participants who displayed both values. According to the commonly-used table of critical values for correl-ation coefficients [52] using n-2 degrees of freedom and 5% type I error, the correlation coefficient that is considered statistically significant for the total sample in the present study is $r = 0.195$ ($n = 249$). The same critical correlation coefficient for only the participants who completed the dietary recall in the present study is still $r = 0.195$ ($n = 97$). These critical r-values indicate that the statistical interpretations of the composite correlation coefficients presented in Table 2, regardless of the smaller sample of dietary recalls, may be considered the same. Therefore, we believe that the smaller sample size of n = 97 for completed dietary recalls is still acceptable for addressing the research questions in this study.

Another potential limitation exists regarding sample size and the interpretations of iron biomarkers and dietary intakes for females. Since n = 24–31 samples were collected for iron biomarkers and dietary intakes, the critical r-values for these correlations are r = 0.349–0.423 [52]. However, we believe that the moderate correlations between sTfR concentrations and athletic performance, as well as the moderate correlations between athletic performance and dietary intakes, in the adolescent female athletes in the present study should not be ignored. Not only are children and adolescents a protected human subject population making it difficult to collect these data, but also adolescent female athletes may be considered an under-studied population. Together with the exploratory, correlational premise of the present study, we believe that these moderate correlations emphasize the need to collect additional data in adolescent female athletes in future studies to improve nutritional recommendations for this at-risk population.

Conclusions

In conclusion, sTfR was moderately related to athletic performance (VJ, PA, LC, and 20YD) in the adolescent female athletes, possibly reflecting an increased rate of erythropoiesis during their growth spurt. However, after removing the collinear influence of dietary iron intake, relationships between sTfR and VJ and 20YD were eliminated, suggesting that improving dietary iron intake may improve lower-body power and speed in adolescent female athletes. The pre-adolescent male athletes showed significant, but negligible to low, relationships between Hb and athletic performance. After removing potential collinear influences of both growth and development (age and height) and dietary intakes (energy and carbohydrates), the relationships between Hb and athletic performance remained unaffected. From a more global perspective, perhaps the negligible to moderate correlations between iron status biomarkers (sTfR and Hb) and anaerobic performance in both male and female youth athletes reflect the subtle contributions of oxygen to exercise that is not exclusively anaerobic [53]. Interestingly, the fact that the adolescent females and pre-adolescent males exhibited different iron biomarker correlations, despite being at the same chronological age, suggested that iron status biomarkers may be more maturity- dependent than age-dependent. The largest differences between sexes in the present study included 32 to 40% greater upper-body strength (PPU) and muscle mass (arm eCSA), dietary protein and iron intakes, and ferritin concentrations for the young males. Based on these comparisons, we would recommend increasing dietary protein and iron intakes in young female athletes of this age. Nevertheless, these hypotheses need to be

experimentally tested to clarify the underlying physiological relationships involving iron status biomarkers in pre-adolescent and adolescent athletes. Specifically, future studies should examine the effects of increasing dietary iron intake on ferritin, sTfR, and Hb concentrations, as well as athletic performance, in adolescent female athletes.

Abbreviations

20YD: 20-yard Dash; AGP: Alpha 1-acid Glycoprotein; BF%: Percent Body Fat; BJ: Broad Jump; eCSA: Estimated Cross-sectional Area; ELISA: Enzyme-linked Immunosorbent Assay; Hb: Hemoglobin; LC: L-cone; PA: Pro-agility; PARQ+: Physical Activity Readiness Questionnaire for Everyone; PPU: Power Push Up; sTfR: Soluble Transferrin Receptor; VJ: Vertical Jump

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Competing interests

The authors declare that they have no competing interests.

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Red meat and dietary iron intakes are associated with some components of metabolic syndrome: Tehran Lipid and Glucose Study

Abstract

Background: This study was conducted to investigate whether the daily consumption of haem, non-haem, total iron and red meat can affect the occurrence of metabolic syndrome (MetS) and its components.

Methods: Eligible adults (n = 4654) were selected from among participants of the Tehran Lipid and Glucose Study with an average follow-up of 3.8 years. Dietary intakes were assessed using a valid and reliable semi-quantitative food frequency questionnaire. Anthropometrics and biochemical variables were evaluated at baseline and follow-up examinations. The occurrence of MetS and its components were assessed in relation to haem, non-haem, total iron and red meat intakes.

Results: There was no relationship between different types of dietary iron and red meat intakes and the incidence of MetS in the Tehranian population. Risk of hypertension decreased from quartiles 1 to 4 for haem iron (HR: 1.00, 0.92, 0.81, 0.80, $P_{trend} < 0.01$) and red meat intake (HR: 1.00, 0.89, 0.84, 0.77, $P_{trend} < 0.01$). The association between hyperglycemia and the fourth quartile of total iron intake was significant (HR = 1.98, 95% CI 1.08–3.63); and the risk of high triglyceride appeared to increase in higher quartiles of total iron intake (HR: 1.00, 1.17, 1.49, 1.75, $P_{trend} = 0.01$) compared to lower quartiles.

Conclusion: Our study suggests a potentially protective relationship of haem and moderate red meat intake against development of high blood pressure; and higher intake of total iron is related to hyperglycemia and high triglyceride.

Keywords: Red meat, Dietary iron intake, Metabolic syndrome

Background

The prevalence of metabolic syndrome (MetS) has increased in older ages and 25% of adults suffer from MetS [1]. MetS is a cluster of metabolic abnormalities including hyperglycemia, dyslipidemia, high blood pressure (BP) and central obesity, which increases the risk of type 2 diabetes [2], cardiovascular disease [3], specific cancers [4] and mortality [5]. The close relationship between MetS and diet has been approved [6] and there is concern on finding which nutrients or foods reduce or increase the risk of MetS.

The results of previous studies indicate that meat consumption (especially red meat) is associated with an increased risk of MetS, which may be related to the high level of iron and saturated fat in meat [2, 7–9]. Some studies have shown an association between the ferritin levels in serum and MetS [10–12]. Iron overload is specified by an increment in the serum ferritin levels [13], and some studies have reported that meat or heme iron intake is related to the serum ferritin [14, 15]. A few studies have demonstrated the association between consumption of dietary iron and MetS, which could potentially address the causative character of the association between iron metabolic markers and MetS [16–18]. Iron

is present in foods in a heme or non-heme form, which present differences in absorption, bioavailability, metabolism and food sources. Heme iron is more efficiently absorbed than non-heme iron as nearly 25% heme iron and 5% non-heme iron from diet absorbed by intestine [19]. The Iranian diet is known to be plant-based, which implies a low bioavailability of dietary iron because of high contribution of non-haem iron in diet [20].

As far as we know, there is no study about the association between dietary iron and MetS in the Middle east; hence, the aim of the current study was to determine the association of haem, non-haem, total iron and red meat intakes with MetS and its components in an Iranian population.

Methods

Study population

Subjects of this cohort study were selected from participants of the Tehran Lipid and Glucose Study (TLGS), a population-based prospective study performed to determine the risk factors for non-communicable diseases in a sample of residents from District 13 of Tehran, the capital of Iran (20). The first examination survey was performed from 1999 to 2001 on 15,005 individuals aged ≥ 3 years, using the multistage stratified cluster random sampling technique, and follow-up examinations were conducted every 3 years; 2002–2005 (survey 2), 2005–2008 (survey 3), 2008–2011 (survey 4), and 2012–2015 (survey 5) to identify recently developed diseases.

Of individuals participating in surveys 3 and 4, respectively 3682 and 7897 subjects were randomly selected for dietary assessment. For the current study, a total of 8177 adult men and women aged ≥ 18 years with available dietary, biochemical and anthropometric data were selected as the baseline population and followed until survey 5 (participants entered at surveys 3 and 4 had respectively followed two times and one time for the outcome measurements). Of these participants, we excluded pregnant or lactating women, those with under- or over-report of energy intake (< 800 or ≥ 4200 kcal/day) (n = 547) and also subjects with prevalent MetS (n = 2325) at baseline. Finally, after excluding participants missing any follow up data (n = 632), 4654 subjects remained and entered the analysis. Other separate lines of exclusion were performed for components of metabolic syndrome, including high triglyceride, low high density lipoprotein cholesterol (HDL-C), abdominal obesity, high fasting blood sugar (FBS) and high BP (Fig. 1).

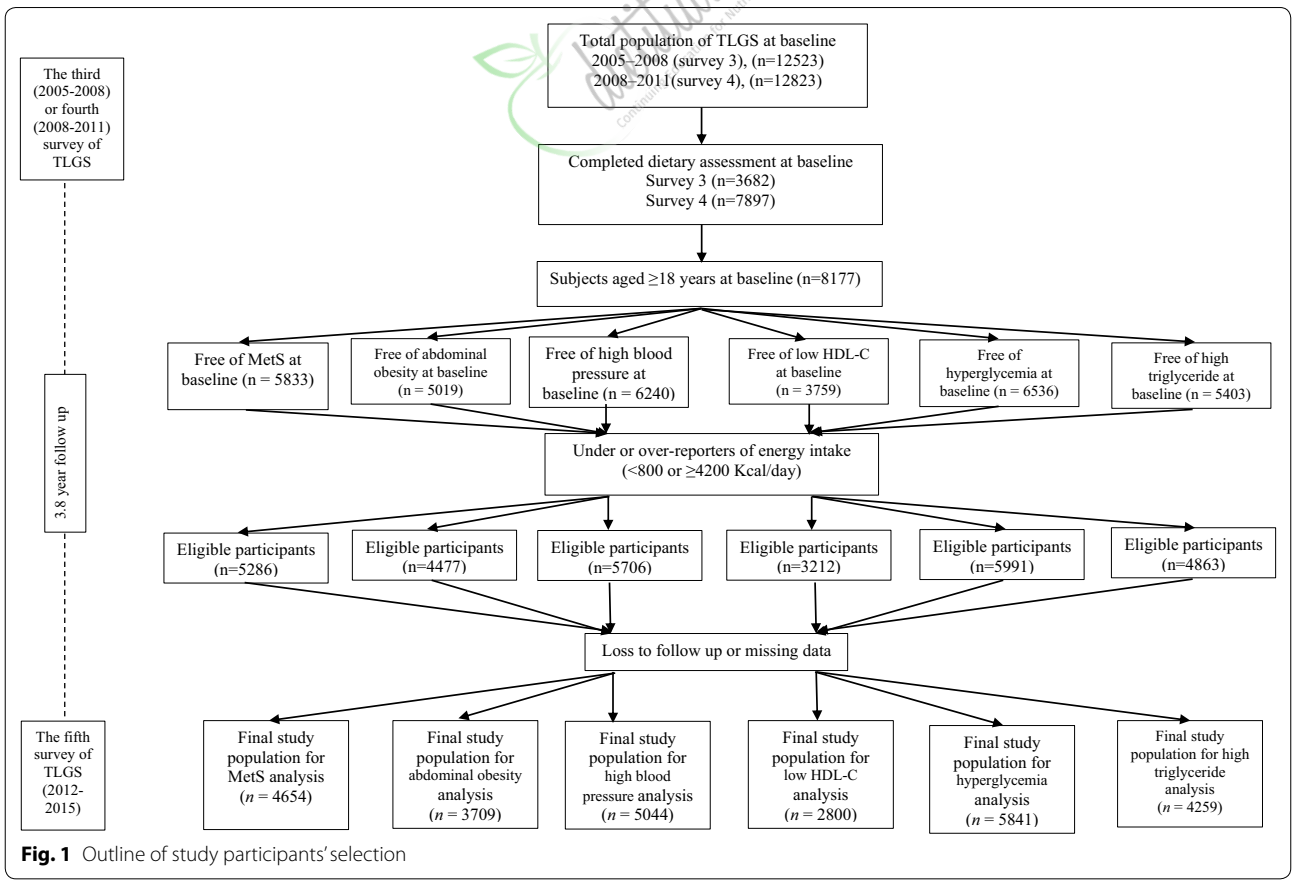

Fig. 1 Outline of study participants' selection

All participants signed a written informed consent form before taking part in this investigation. The study was implemented based on the Declaration of Helsinki and the study protocol was accepted by the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All methods were performed in line with their relevant guidelines and regulations.

Dietary intake measurements

Dietary assessment was performed by a valid and reliable 168-item semi-quantitative food frequency questionnaire (FFQ); expert dietitians collected information on the intake of standard serving sizes of a list of foods, through face-to-face personal interviews. The consumption frequency of each food item on a daily, weekly, or monthly basis was converted to daily intakes; portion sizes were then converted to mass (in grams), using household measures. Since the Iranian food composition table (FCT) is incomplete, the United States Department of Agriculture (USDA) FCT was used to analyze foods [21]. Red meat was defined as the sum of beef, lamb, organ meats (kidney, beef liver and heart) and processed meats (sausages and hamburger). Haem iron was estimated as 40% of the total iron from poultry, fish, beef, lamb, organ meats and processed meats [22]. Non-haem iron was calculated as the difference between total iron and haem iron.

Physical activity

Physical activity level was evaluated using the Persian-translated modifiable activity questionnaire with high reliability and relative validity. Information on the time and frequency of light, moderate, high, and very high severity activities were collected according to the list of usual activities of daily life over the past year. Physical activity levels were reported based on the metabolic equivalent-h/week (Met/h/week) [23–25].

Anthropometric measurements

Weight was measured to the nearest 100 g, using digital scales (Seca 707), while the subjects were minimally clothed and not wearing shoes. Height was measured to the nearest 0.5 cm by a tape measure, in standing position with shoulders in normal alignment and without shoes. Waist circumference (WC) was taken at the end of normal expiration, over light clothing, with a non-flexible tape meter at the level of the umbilicus without any pressure to body surface; measurements were recorded to the nearest 0.1 cm.

Laboratory assays

Blood samples were drawn into vacutainer tubes from subjects who were in sitting position between 7:00 to 9:00 a.m., after a 12–14 h overnight fast. Blood samples were centrifuged within 30 to 45 min of collection. All biochemical analyses were performed using a Selectra 2 auto-analyzer at the TLGS research laboratory on the day of blood collection. FBS concentration was measured by the enzymatic colorimetric method using the glucose oxidase technique. HDL-C concentration was assessed after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. triglyceride level was determined by enzymatic colorimetric tests using glycerol phosphate oxidase and triglyceride kits. Assay performance was monitored once in every 20 tests, using lipid control serum, Percinorm (normal range) and Percipath (pathological range), where applicable (Boehringer Mannheim; catalog no. 1446070 for Percinorm and 171778 for Percipath). A lipid standard (Cfas, Boehringer Mannheim; catalog no. 759350) was used to calibrate the Selectra 2 auto-analyzer on each day of the laboratory analysis, and all samples were analyzed only when the internal quality control met the standard criteria. Inter- and intra-assay coefficients of variations were both 2.2% for serum glucose and 1.6% and 0.6% for triglyceride, respectively [26].

Definitions

Individuals with three or more of the following criteria for MetS were considered as unhealthy phenotypes based on the Iranian modified National Cholesterol Education Program/Adult [27, 28]: (1) Abdominal obesity (WC $\geq$ 95 cm in men and women); (2) BP $\geq$ 130/85 mmHg or antihypertensive drug treatment; (3) HDL-C $<$ 1.30 mmol/l ($<$ 50 mg/dl) in women, and $<$ 1.04 mmol/l ($<$ 40 mg/dl) in men or receiving drug treatment; (4) FBS $\geq$ 6.11 mmol/l ($\geq$ 110 mg/dl) or drug treatment for hyperglycemia; (5) triglyceride $\geq$ 1.70 mmol/l ($\geq$ 150 mg/dl) or drug treatment.

Statistical analyses

Statistical analyses were carried out using the Statistical Package for Social Sciences (version 21.0; SPSS). A two-tailed P value $<$ 0.05 was used to determine statistical significance. All types of iron and red meat intakes were adjusted for total energy intake using the residual model [29]. We used a Chi square test for qualitative variables and the one way ANOVA for quantitative variables to compare the characteristics across quartiles of the average energy-adjusted daily intake of total iron. In case of non-normal nutritional and biochemical variables (triglyceride concentration), log-transformed values were

used for statistical analysis. The hazards ratio (HR) and 95% confidence interval of incident MetS and its components were assessed using multivariable Cox proportional hazard regression models. The incidence of MetS or its components during the follow up period were considered as dichotomous variables (yes/no) in the models. Different types of dietary total iron (Q1 < 13.87, Q2: 13.87–16.03, Q3: 16.04–19.85, Q4 > 19.85 mg/day), haem (Q1 < 0.26, Q2: 0.27–0.39, Q3: 0.40–0.57, Q4 > 0.57 mg/day), non-haem (Q1 < 13.45, Q2: 13.46–15.51, Q3: 15.52–19.17, Q4 > 19.17 mg/day) iron and red meat (Q1 < 30.50, Q2: 30.51–36.33, Q3: 36.44–49.91, Q4 > 49.91 g/day) intake were categorized into quartiles, given the first quartile as the reference. The survival time for censored individuals was calculated as the interval between the first and last observation dates. The event date for the incidence of MetS and its components was considered as the mid-time between the date of the follow up visit at which the events were diagnosed for the first time, and the most recent follow up visit preceding the diagnosis. Study participants were censored due to loss to follow-up, the end of the observation period or death. The median of each quartile was used as a continuous variable to assess the overall trends of HRs across quartiles of dietary iron and red meat intakes in the Cox proportional hazard regression models. The proportional hazard assumption of multivariate Cox models were assessed using Schoenfeld's global test of residuals.

The confounders were selected based on literature, also each confounder was included in the univariable Cox regression model. A two-tailed P value < 0.20 was used for determining inclusion in the model. The Cox regression models were adjusted for several potential confounders; the analyses were adjusted for sex, age, BMI, education levels (> 14 and ≤ 14 years), smoking (never smoked, past smoked, and current smoker), physical activity (continuous), dietary iron supplements, fiber (gr/1000 kcal), saturated fat (percentage of energy), magnesium, vitamin C and total energy intake; in models for estimating HR of high BP and high triglyceride, sodium (continuous) and total fat (percentage of energy) have been added, respectively. In Cox regression models where haem iron was a predictive variable, non-haem iron was included in the model as an adjustment variable and vice versa.

Results

Characteristics of the participants

General characteristics of the study population across quartiles of total dietary iron intake are presented in Table 1. Subjects in the lower quartiles of total iron intake were younger; also, they had a higher percentage of smokers and lower BMI. Dietary intakes including carbohydrate, protein, fiber, vitamin C and magnesium were significantly different among quartiles of total iron intake and also the prevalence of MetS and its components were significantly different among quartiles of total iron intake.

Association of dietary iron intake with MetS

After an average follow-up of 3.8 years, new-onset of MetS was developed in 1106 participants. The association between MetS development and the quartiles of iron (total, haem and non-haem) and red meat intake are presented in Table 2. In the crude model, subjects in the upper quartile of total and non-haem iron intake had a higher risk of incident MetS than those in the lowest quartile ($P_{trend} < 0.05$). High consumption of red meat was associated with a lower MetS risk ($P_{trend} < 0.01$); However, when potential confounders were considered, the statistical significance of crude models disappeared.

Association of dietary iron intake with components of MetS

HR and 95% confidence interval of the MetS components for energy-adjusted quartiles of iron and red meat intakes are shown in Table 3.

Risk of hypertension decreased from quartiles 1 to 4 for haem iron (HR (95% CI) 1.00, 0.93 (0.81, 1.07), 0.82 (0.71, 0.95), 0.81 (0.70, 0.94), $P_{trend} < 0.01$) and red meat intake (HR (95% CI) 1.00, 0.90 (0.79, 1.02), 0.82 (0.71, 0.96), 0.76 (0.65, 0.87), $P_{trend} < 0.01$).

With respect to quartile one, participants in the fourth quartile had a higher risk of hyperglycemia (HR = 1.98, 95% CI 1.08–3.63); and the risk of high triglyceride appeared to increase significantly in higher quartiles of total iron intake (HR (95% CI) 1.00, 1.22 (0.96, 1.56), 1.62 (1.14, 2.29), 1.89 (0.80, 4.04), $P_{trend} = 0.01$) compared with the lower quartiles.

Discussion

The current investigation was a prospective cohort study, evaluating the association of dietary iron and red meat intakes with MetS or its components. Our results suggested that there was no relationship between any type of dietary iron and red meat intake, and the incidence of MetS in the Tehranian population. Incidence of hypertension decreased with high intake of haem iron and red meat intake, after adjusting for several confounders, and total iron intake was positively associated with hyperglycemia and high triglyceride.

Red meat, dietary iron intakes and MetS

This non-significant association between MetS and red meat intake was similar to previous results in the Asian population but not Western population. Consistent with our study, a recent meta-analysis study showed an inverse but non-significant association between MetS

Table 1 Baseline characteristics of the study population, across the quartiles of total iron intake in the Tehran Lipid and Glucose Study

Characteristic	Total iron consumption (mg/day)				
	Q1	Q2	Q3	Q4	P
Baseline age (years)	38.19 ± 14.1[a]	41.54 ± 14.4	42.30 ± 14.6	40.42 ± 14.1	< 0.01
Women, % (n)	27.4 (887)	24.0 (778)	22.3 (722)	26.4 (856)	< 0.01
Current smokers (%)	24.6	21.6	23.5	20.0	< 0.01
Education level (%)†	22.5	27.4	30.1	32.7	< 0.01
Physical activity (MET/min/week)	584 ± 891	536 ± 837	553 ± 805	546 ± 834	0.34
BMI (Kg/m^2)	25.2 ± 4.9	25.9 ± 4.8	25.8 ± 4.8	25.7 ± 4.9	< 0.01
Energy intake (kcal/day)	2440 ± 715	2076 ± 664	2346 ± 724	2530 ± 698	< 0.01
Carbohydrate (% of energy)	53.8 ± 6.7	58.6 ± 5.7	60.5 ± 6.5	60.5 ± 10.5	< 0.01
Protein (% of energy)	13.5 ± 2.2	14.4 ± 2.7	14.9 ± 3.9	15.0 ± 9.4	< 0.01
SFA (% of energy)	12.1 ± 5.2	10.0 ± 2.3	9.1 ± 2.9	10.0 ± 21.4	< 0.01
Fiber (g/1000 kcal)	13.5 ± 4.5	18.0 ± 6.2	21.5 ± 8.9	20.2 ± 11.0	< 0.01
Vitamin C (mg/day)	59.8 ± 32.5	67.3 ± 35.0	74.8 ± 42.0	81.1 ± 50.0	< 0.01
Magnesium (mg/day)	159 ± 30	181 ± 34	198 ± 43	202 ± 44	< 0.01
Total iron (mg/day)	12.2 ± 4.0	14.9 ± 4.7	18.8 ± 5.9	23.6 ± 7.9	< 0.01
Haem iron (mg/day)	0.49 ± 0.29	0.53 ± 0.32	0.51 ± 0.34	0.50 ± 0.30	< 0.01
Non-Haem iron (mg/day)	10.7 ± 1.7	13.2 ± 1.3	14.7 ± 2.2	15.8 ± 3.4	< 0.01
Red meat (g/day)	30.5 ± 19.6	32.6 ± 18.4	31.9 ± 20.4	31.1 ± 18.0	< 0.01
Metabolic syndrome (%)	19.7	26.0	28.0	26.1	< 0.01
Abdominal obesity (%)	34.0	41.0	43.8	40.5	< 0.01
High blood pressure (%)	20.1	25.5	27.9	25.6	< 0.01
Low HDL-C (%)	62.7	59.5	56.7	51.2	< 0.01
Hyperglycemia (%)	14.3	20.6	24.5	25.5	< 0.01
High triglyceride (%)	31.3	38.3	39.2	34.9	< 0.01

Q quartiles of total iron consumption, *MET* metabolic equivalent, *BMI* body mass index, *MUFA* mono-unsaturated fatty acids, *PUFA* poly-unsaturated fatty acids, *SFA* saturated fat

[a] Values are mean ± SD unless otherwise listed

† Educational level ≥ 14 years

and red meat intake [RR = 0.91 (95% CI 0.82, 1.00)] in the Asian population. However, the Western population had a 33% higher risk of MetS in the upper category of red meat intake compared to those in the lowest intake category. This discrepancy in the effect of red meat intake may be substantially due to lower consumption of red meat in our study population (total meat intake in the highest quartile was less than 2 serving/day) and also, in the Asian population compared to the Western [9, 30]. According to the OECD (Organization for Economic Cooperation and Development), consumption of meat is significantly low in Asian countries [31].

In line with our study, no association was reported between haem iron intake and the risk of MetS in a recent cross-sectional study in people Republic of China [17]. This result was inconsistent with studies on Western populations [16, 32], which showed that intake of haem iron is associated with MetS. The amount of haem iron intake might explain this inconsistency. Compared to haem iron intake of the Western population, the average intake of heam iron in the current study was lower (haem median dietary iron intake was 0.39 mg/day) [16, 32–34]. Heam iron is a stronger predictor of serum ferritin compared to non-haem iron. Elevated serum ferritin levels have appeared as a characteristic in individuals with MetS [10, 34].

Red meat, dietary iron intakes and components of MetS

A negative association was observed between high BP, and haem iron and red meat intake. Kim et al. [35] reported that Korean children and adolescents who consumed more than 5 servings of red meat and chicken per week, had a lower prevalence of high blood pressure compared to those who consumed less than 5.

The BOLD Study showed that systolic blood pressure reduced after the intake of DASH (Dietary Approaches to Stop Hypertension) diet with 153 g of lean beef (main

Table 2 Hazard ratios (95% CI) of metabolic syndrome across energy-adjusted quartiles of iron (total, haem and non-haem) and red meat intake in adult participants of the Tehran Lipid and Glucose Study

Characteristic	Quartiles of dietary iron and red meat intake				
	Q1	Q2	Q3	Q4	P_{trend}
Total iron (mg/day)	< 13.87	13.87–16.03	16.04–19.85	> 19.85	
Crude	1.00 ref.	1.14 (0.97–1.34)	1.40 (1.19–1.65)	1.18 (0.98–1.43)	0.02
Model 1[a]	1.00 ref.	0.97 (0.79–1.19)	1.10 (0.81–1.49)	2.04 (0.97–4.28)	0.22
Haem iron (mg/day)	< 0.26	0.27–0.39	0.40–0.57	> 0.57	
Crude	1.00 ref.	0.94 (0.80–1.11)	0.85 (0.72–1.01)	0.84 (0.71–1.00)	0.35
Model 1[b]	1.00 ref.	0.90 (0.71–1.14)	0.89 (0.70–1.12)	0.87 (0.67–1.12)	0.30
Non-Haem iron (mg/day)	< 13.45	13.46–15.51	15.52–19.17	> 19.17	
Crude	1.00 ref.	1.10 (0.93–1.30)	1.36 (1.15–1.61)	1.44 (1.21–1.71)	< 0.01
Model 1[c]	1.00 ref.	0.98 (0.78–1.24)	1.16 (0.89–1.52)	1.15 (0.80–1.63)	0.46
Red meat (g/day)	< 30.50	30.51–36.33	36.44–49.91	> 49.91	
Crude	1.00 ref.	0.83 (0.70–0.99)	0.85 (0.72–1.01)	0.69 (0.58–0.82)	< 0.01
Model 1[a]	1.00 ref.	0.86 (0.55–1.26)	0.96 (0.68–1.28)	0.87 (0.56–1.24)	0.43

[a] Adjusted for age, sex, baseline BMI, educational level, smoking status, total energy intake, fiber, saturated fat, sodium, vitamin C and magnesium intakes

[b] Adjusted for age, sex, baseline BMI, educational level, smoking status, total energy intake, fiber, saturated fat, sodium, vitamin C, magnesium, and non-haem iron intakes

[c] Adjusted for age, sex, baseline BMI, educational level, smoking status, total energy intake, fiber, saturated fat, sodium, vitamin C, magnesium and haem iron intakes

source of haem iron) per day, but did not reduce after DASH diets including 113 g or 28 g of lean beef per day [36]. It seems that moderate red meat intake decreased high blood pressure. In addition, subjects classified into total and non-haem iron consumption quartiles showed an ascending trend for intake of fiber from quartiles 1 to 4 for total and non-haem iron. Increasing fiber intake can improve BP [37].

A positive relationship has been shown between total iron intake and incident hyperglycemia, in two cross-sectional and one prospective study [16, 38, 39]. Iron overload can act as a strong pro-oxidant, and cause oxidative stress and damage to tissues such as pancreatic beta cells, which can decrease the synthesis and secretion of insulin, impair insulin signaling, and finally, change glucose metabolism [40, 41]. A direct association between iron intake and the increased risk of hyperglycemia has been previously found [16, 42].

The mechanisms underlying the associations between total iron intake and high triglyceride levels are uncertain. Although, we hypothesize that high intake of iron may lead to increased risk of iron overload and then enhance the generation of inflammation, which can cause insulin resistance and then hyperinsulinaemia; these conditions may reduce insulin-mediated suppression of hormone-sensitive lipase (enzyme responsible for mobilization of triglyceride), which can increase intracellular lipolysis, plasma levels of free fatty acids and their transport to the liver. The increment in the levels of liver free fatty acids motivates triglyceride-rich lipoprotein production [43–48]. Also, our results are along with previous reports showing a relationship between red meat (the main source of iron) intake and high triglyceride levels [8, 16, 45]. In our population non-haem iron had a higher contribution of total iron; as well there was a borderline association between non- haem iron and high triglyceride ($P_{trend} = 0.05$). main source of non-haem in our population is grains (specially refined grains) [20], which reveals that higher intake of non- haem iron is associated with higher intake of simple carbohydrates. It can explain the reason of hyperglycemia [49] and high triglyceride [50].

The present study has important strengths. The comparison of associations with total, haem and non-haem iron provided insight into the role of these different types of iron in the incidence of MetS and its components. The prospective design allowed the estimation of incident disease with less worry about reverse causality between nutrients and outcomes. The evaluation of nutrient consumption from various food sources provided a new vision into the association between disease and nutrients. Limitations, include lack of data on serum levels of iron and total iron binding capacity, and under or over estimations of dietary intakes as an inherent limitation of FFQ. Despite that the nutrients were adjusted for important confounders, some confounders such as CRP were not included.

Conclusion

Our study suggests that a higher consumption of haem iron red meat is negatively associated with elevated blood pressure, and a high intake of total iron is related to hyperglycemia and high triglyceride. Furthermore, the

Table 3 Hazard ratios (95% CI) of metabolic syndrome components across energy-adjusted quartiles of iron (total, haem and non-haem) and red meat intake in adult participants of the Tehran Lipid and Glucose Study (n=7630)

Characteristic	Quartiles of dietary iron and red meat intake				
	Q1	Q2	Q3	Q4	P_{trend}
Total iron					
Abdominal obesity[a]	1.00 ref.	0.74 (0.54–0.95)	1.18 (0.79–1.76)	1.11 (0.34–2.32)	0.68
High blood pressure[b]	1.00 ref.	1.00 (0.90–1.13)	1.13 (0.97–1.37)	1.04 (0.59–1.84)	0.52
Low HDL-C[a]	1.00 ref.	1.02 (0.72–1.44)	1.20 (0.73–1.99)	1.38 (0.31–5.99)	0.07
Hyperglycaemia[a]	1.00 ref.	0.98 (0.81–1.18)	1.07 (0.81–1.42)	1.98 (1.08–3.63)	0.19
High triglyceride[c]	1.00 ref.	1.22 (0.96–1.56)	1.62 (1.14–2.29)	1.89 (0.80–4.04)	0.01
Haem iron (mg/day)					
Abdominal obesity[d]	1.00 ref.	0.82 (0.59–1.14)	0.74 (0.52–1.04)	0.88 (0.62–1.25)	0.44
High blood pressure[e]	1.00 ref.	0.93 (0.81–1.07)	0.82 (0.71–0.95)	0.81 (0.70–0.94)	0.00
Low HDL-C[d]	1.00 ref.	0.67 (0.44–1.01)	0.67 (0.44–1.02)	0.93 (0.61–1.42)	0.99
Hyperglycaemia[d]	1.00 ref.	0.96 (0.77–1.20)	1.04 (0.83–1.30)	0.96 (0.75–1.22)	0.80
High triglyceride[f]	1.00 ref.	0.98 (0.75–1.30)	1.14 (0.87–1.51)	1.16 (0.87–1.55)	0.19
Non-Haem iron					
Abdominal obesity[g]	1.00 ref.	0.73 (0.54–1.00)	0.90 (0.52–1.28)	0.96 (0.60–1.54)	0.58
High blood pressure[h]	1.00 ref.	1.03 (0.92–1.15)	1.13 (0.99–1.31)	1.19 (0.98–1.44)	0.32
Low HDL-C[g]	1.00 ref.	1.01 (0.79–1.28)	1.13 (0.89–1.43)	1.21 (0.95–1.53)	0.64
Hyperglycaemia[g]	1.00 ref.	0.98 (0.79–1.21)	1.03 (0.80–1.33)	1.24 (0.90–1.71)	0.21
High triglyceride[i]	1.00 ref.	1.00 (0.75–1.34)	1.09 (0.95–1.75)	1.48 (0.99–2.74)	0.05
Red meat					
Abdominal obesity[a]	1.00 ref.	0.75 (0.55–1.03)	0.74 (0.53–1.04)	0.82 (0.59–1.14)	0.47
High blood pressure[b]	1.00 ref.	0.90 (0.79–1.02)	0.82 (0.71–0.96)	0.76 (0.65–0.87)	0.00
Low HDL-C[a]	1.00 ref.	0.96 (0.76–1.21)	1.14 (0.91–1.42)	0.95 (0.76–1.19)	0.85
Hyperglycaemia[a]	1.00 ref.	1.03 (0.82–1.30)	1.00 (0.80–1.24)	0.91 (0.72–1.13)	0.23
High triglyceride[c]	1.00 ref.	1.09 (0.81–1.43)	1.07 (0.82–1.48)	1.11 (0.82–1.45)	0.42

[a] Adjusted for age, sex, baseline BMI, educational level, smoking status, total energy intake, fiber, saturated fat, vitamin C and magnesium intakes

[b] Additionally adjusted for sodium

[c] Additionally adjusted for total fat

[d] Additionally adjusted for non-haem iron

[e] Additionally adjusted for sodium and non-haem iron

[f] Additionally adjusted for total fat and non-haem iron

[g] Additionally adjusted for and haem iron

[h] Additionally adjusted for sodium and haem iron

[i] Additionally adjusted for total fat and haem iron

present study stresses the important role of moderate red meat intake on blood pressure.

Abbreviations

MetS: metabolic syndrome; BP: blood pressure; TLGS: Tehran Lipid and Glucose Study; HDL-C: high density lipoprotein cholesterol; FFQ: food frequency questionnaire; FCT: food composition table; USDA: United States Department of Agriculture; Met: metabolic equivalent; WC: waist circumference; FBS: fasting blood sugar; MUFA: mono unsaturated fatty acids; PUFA: poly unsaturated fatty acids; SFA: saturated fatty acids.

Competing interests

All the authors declare that they have no competing interests.

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Iron-related dietary pattern increases the risk of poor cognition

Abstract

Introduction: High iron intake has been shown to be associated with poor cognition. We aimed to examine the association between iron-related dietary pattern (IDP) and cognitive function in Chinese adults.

Method: Longitudinal study data from the China Health and Nutrition Survey (CHNS) during 1991–2006 were used ($N = 4852$, ≥55 years old). Dietary intake was obtained from a 3-day food record during home visits. Reduced rank regression was used to construct IDP with iron intake as a response variable. Cognitive function was assessed in 1997, 2000, 2004 and 2006. Multivariable mixed linear regression and logistic regression were used in the analyses.

Results: IDP was characterised by high intake of fresh vegetable, wheat, legume, beverage, offal, rice and whole grain. High IDP intake was associated with poor cognition. In fully adjusted models, across the quartiles of IDP, the odds ratio (95% CI) for poor cognitive function were: 1.00, 1.06 (0.86–1.30), 1.24 (0.99–1.54), and 1.50 (1.17–1.93), respectively. There was a borderline significant interaction between IDP and meat intake (p interaction 0.085). The association between high IDP and poor cognition was only observed among those with no or low intake of meat. With the adjustment of carbohydrate or iron intake, the IDP and cognition association became non-significant. IDP was positively associated with lead intake. The association between IDP and poor cognition was partly mediated by lead intake.

Conclusions: Iron-related dietary pattern is associated with poor cognition in Chinese adults, partly due to high intake of carbohydrate, iron and lead.

Keywords: Cognitive function, Dietary pattern, Lead intake, Chinese, Adults

Introduction

Worldwide, dementia was estimated to affect 35.6 million people in 2010, and this number is expected to reach 115.4 million by 2050 [1]. It affects approximately 9.5 million adults aged 60 years and above in China [2]. As there is still no effective treatment to delay the progression of dementia, identifying modifiable risk factors other than hypertension, diabetes and stroke that can be used in the early prevention is urgently needed. The role of diet on cognitive decline and dementia has increasingly attracted attention [3, 4].

Many nutrients (e.g. fiber, carbohydrate, protein, docosahexaenoic acid (DHA)) and foods (e.g. alcohol, nuts, fish) have been assessed for their potential effects on cognition [4]. The traditional single nutrient and disease association approach does not consider the possible synergy or interaction between nutrients. The use of dietary pattern using either a priori (e.g. healthy eating index, Mediterranean diet score) or a posterior (e.g. factor analysis, cluster analysis) approach is becoming popular in nutritional epidemiology [5]. While a high proportion of studies uses factor analysis to construct dietary pattern, there is also an increasing number of studies using Reduced Rank Regression (RRR) to construct dietary pattern [6]. The advantage of RRR method is that it uses biomarkers or nutrients as intermedia responses so that it can explore the potential mediation effects of diet on disease [6].

Similar to the studies in Western countries [7], several studies in Asia suggested a link between dietary patterns and cognition among older adults. For example, in Korea, using RRR method with responses regarding vitamin B6, vitamin C, and iron intakes, a dietary pattern characterized by high intake of seafood, vegetables, fruits, bread, snacks,

soy products, beans, chicken, pork, ham, egg, and milk was found to be associated with a decreased risk of mild cognitive impairment [8]. A diet with high intakes of vegetables, soy products, fruit, and fish may have a beneficial effect on cognitive function in older Japanese people using the similar approach [9].

There is increasing evidence from epidemiological studies suggesting the link between high iron levels and chronic diseases including dementia. Basic science also demonstrates that iron accumulation in the brain increases with age [10]. In the Western countries, higher dietary iron intake has been found to be associated with higher risk for Parkinson disease [11, 12]. In many countries, it has been shown that high iron levels increases the risk for diabetes [13], which is linked to cognitive impairment [14]. Despite these initial findings, the association between iron intake and cognition among older adults is inconsistent, as both positive [15, 16] and negative [17] associations have been reported.

Additionally, studies have shown that the modern Western diet with higher simple carbohydrates and saturated fat intake is correlated with cognitive impairment [18]. Conversely, clinical intervention studies of very low carbohydrate (5–10% of total calories) consumption shows improved verbal memory in the elderly [19] as well as improved overall cognitive performance in adults with type 2 diabetes [20, 21].

Furthermore, cognitive impairment has been found to be associated with both occupational and non-occupational lead exposure as indicated by blood and bone lead [22]. In the general population, diet is a major source of heavy metals in human body [23]. However, the number of studies on the association between dietary pattern and heavy metal contamination is limited. Lead levels have been implicated in impaired cognitive function across the lifespan [24]. It is unknown whether the association between dietary pattern and cognitive impairment is mediated by heavy metal contamination. We have recently reported a positive association between high iron intake and poor cognition among Chinese adults [17]. However, given the possible interaction effects between nutrients, it is important to look at overall dietary pattern rather than a single nutrient. Iron intake could be marker of other unmeasured factors. In China, the major source of dietary iron is from plant-based food. At the same time, lead contamination on plant-based food is of concern. For example, data from a large surveillance system in China suggested that 6.4% of cereal grain and pulse samples had lead concentration exceeding the maximum level of 0.2 mg/kg [25]. Lead intake has been found to be positively associated with all-cause mortality [26]. Plant-based food is also the major source of carbohydrate. Furthermore, exposure to lead has been consistently associated with cognitive impairment across the lifespan [27, 28] and has been demonstrated to reduce cognitive function in older adults [29, 30]. Thus, in the current study, we aimed to assess the iron related dietary pattern using RRR method and cognition among Chinese adults attending China Health and Nutrition Survey (CHNS). The second aim of the study was to test whether the association between the dietary pattern and cognition was mediated by lead or carbohydrate intake.

Methods

Study design and study sample

This study used repeated measurements of dietary intake and cognitive function over 15 years since 1991, from the CHNS [31, 32].The CHNS study is an ongoing open prospective household-based cohort study conducted in nine provinces covering both urban and rural areas spanning across Northern to Southern China. Nine waves of data collection (i.e. 1989, 1991, 1993, 1997, 2000, 2004, 2006, 2009, and 2011) have been conducted. Cognitive screen tests were conducted among those above age 55 years in 1997, 2000, 2004 and 2006 surveys. In total, 4852 participants attended the cognitive screen tests between 1997 and 2006 (Additional file 1: Figure S1). Participants who completed at least one cognitive screen test were included in the analysis. Of these participants, 3302 attended the screen test in at least two surveys. The survey was approved by the institutional review committees of the University of North Carolina (USA) and the National Institute of Nutrition and Food Safety (China). Informed consent was obtained from all participants. The response rate based on those who participated in 1989 and remained in the 2006 survey was > 60%.

Outcome variable: cognitive function

The cognitive screening items used in CHNS included a subset of items from the Telephone Interview for Cognitive Status–modified [33]. The screening test was conducted face-to-face during home visit. The screening included immediate and delayed recall of a 10-word list (score 10 for each), counting backward from 20 (score 2), and serial 7 subtraction (score 5). A total verbal memory score was calculated as the sum of the immediate and delayed 10-word recall. The total global cognitive score ranges from 0 to 27. A high cognitive score represents a better cognition. The cognitive function test started with the immediate recall of a 10-word list. The interviewer (i.e. trained health worker) read ten words at a speed of 2 s per word. The participants were given 2 min to memorize the ten words. For each correct recalled word, a score of 1 was given. The participants were then asked to count back from 20 to 1. If the participants made a mistake in the first try, a second chance was given. A score of 2 was given to those answered correctly in the first try, or 1 in the second try. After the

count test, the participants were asked to do five consecutive subtractions of 7 from 100. Each correct subtraction was given a score of 1. Finally, the participants were asked to recall the 10-word list tested before. Each recalled word was given a score of 1. In our study, we choose the first quintile of the cognitive function test score as poor cognitive function, which corresponds to a global cognitive function score cut off of < 7. The cutoff was selected based on a study in Shanghai which showed that the prevalence of mild cognitive impairment among people aged 60 and above was 20% [34].

Exposure variables: Iron related dietary pattern (IDP) and lead

Iron related dietary pattern

Individual food intake was recorded on three consecutive days by a trained investigator at each wave. In the 3-day dietary survey, foods and condiments in the home inventory, foods purchased from markets or picked from gardens, and food waste were weighed and recorded. Nutrients intakes including iron and carbohydrate were calculated based on the average of 3-day food consumption data using the Chinese Food Composition Table [35] . The dietary assessment method has been validated for energy intake [36]. Based on similar nutrient profiles or culinary use, food intake were collapsed into 35 food groups, and average food intake for individuals (gram/day) calculated for each wave. Soft drinks, fruit juice and tea are categorised as beverage. The food groups are similar to the food items used in a validated food frequency questionnaire used in a 2002 Chinese national nutrition survey. The detailed description of the dietary measurement has been provided in the previous publication [31].

IDP was constructed using RRR analysis with the intakes of 35-collapsed food groups as input variables. PROC PLS statement in SAS (SAS Institute Inc., Cary, North Carolina) was used to conduct RRR analysis using iron intake as the response variable [6]. As there was only one response variable, one iron-related dietary pattern was extracted. IDP scores were calculated as the sum of the products of the factor loading coefficients and standardized daily intake of each food group associated with the pattern. We calculated a cumulative average IDP score at each time period to reduce variation within individuals and to represent long term habitual intake [37]. For example, the 1991 intake was used for the follow-up between 1991 and 1993, the average of the 1991 and 1993 intake was used for the follow-up between 1997 and 2000, and so on. Details on cumulative average IDP are illustrated on Additional file 1: Figure S1. In the sensitivity analysis, we also assessed the association between most recent IDP and cognitive function. In sensitivity analyses, we excluded 1991 and 1993 IDP as cognitive function test was not conducted in these surveys. As the main findings did not change, we decided to include 1991 and 1993 dietary data in our analysis.

Lead

Dietary lead of each participant was estimated based on the food intake described above and calculated using published food lead concentration data (mean lead in each food category) from Jiangsu Province (one of the nine provinces in CHNS) [23]. The lead concentration (μg/d) table was based on lead measurements in 2077 food samples from 23 food categories during 2007–2010.

Covariates

Height, weight, and blood pressure were measured at each wave. Overweight/obesity was defined as BMI ≥24 kg/m^2 [38]. Hypertension was defined as systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg, or having known hypertension.

The following constructed sociodemographic variables were used: education (low: illiterate/primary school; medium: junior middle school, and high: high middle school or higher), per capita annual family income (recoded into tertiles as low, medium and high), urbanization levels [31] (recoded into tertiles as low, medium and high).

Physical activity level (Metabolic Equivalent of Task, (MET)) was estimated based on self-reported daily activities (including occupational, domestic, transportation, and leisure time physical activity) and duration using a Compendium of Physical Activities [39]. Smoking status was categorized into non-smokers, ex-smokers and current smokers. Alcohol drinking was categorized as yes or no. Self-reported diabetes and stroke were coded as yes or no.

Statistical analysis

Cumulative mean IDP score was recoded into quartiles. The chi square test was used to compare differences between groups for categorical variables and ANOVA for continuous variables. We use mixed effect model in Stata to assess the association between IDP and cognitive function. A negative regression coefficient represents cognitive function decline. Four multivariable models were used: model 1 adjusted for age, gender and energy intake; model 2 further adjusted for intake of fat, smoking, alcohol drinking, physical activity, income, urbanization, and education; model 3 further adjusted for BMI and hypertension. The fourth model included lead/carbohydrate/iron intake in model 3. It was used to test whether the association between IDP and cognition was mediated by lead or carbohydrate intake by comparing the effect estimated before and after the adjustment of lead, carbohydrate or iron. We also excluded those with a global cognitive function score ≤ 4 and further adjusted for diabetes and stroke. The variables included in

the multivariable models were known to be associated with cognition including socioeconomic status, lifestyle factors and chronic conditions. We chose these variables as covariates because they are both associated with food intake and importantly with cognitive function. Scatter plots were used to visually present the association between IDP and lead intake in 1997, 2000, 2004 and 2006 surveys.

To assess the association between cumulative IDP and the risk of poor cognitive function, we used mixed effect logistic regression adjusting for covariates the same as model 3 mentioned above. In sensitivity analyses, we also stratified our analysis by total meat intake (including pork, beef and poultry, above or below 50 g/d). To test the interaction between IDP and BMI, hypertension, meat intake and income on the association with poor cognition, a product term of each of the two variables was put in the regression model. All the analyses were performed using STATA 15.1 (Stata Corporation, College Station). Statistical significance was considered when $p < 0.05$ (two sided).

Results

IDP derived using RRR methods with iron as a response variable was characterized by high loadings of fresh vegetable, wheat, legume, beverage (i.e. soft drinks, fruit juice and tea), offal, rice and whole grain (Fig. 1). The pattern explained 36.4% of the variation of iron intake. The intake of offal was low between 1991 and 2006 (mean 3.1 g/d, SD 15.4, more than 90% were non-consumers during the 3-day survey). The intake of rice, wheat, legume and fresh vegetable contributed 25.5, 25.0, 1.2 and 15.7% of the total iron intake, respectively.

Table 1 shows the sample characteristics among participants attending the first cognitive function test by quartiles of IDP. Across the quartiles of IDP, the intake of energy, protein, fat, carbohydrate, wheat, rice and fresh vegetable increased. However, there was no difference of fruit intake across quartiles of IDP. IDP was positively associated with smoking, alcohol drinking and physical activity level but inversely associated with age. The mean cumulative intake of iron in the high quartile of IDP was 31.0 mg (SD 12.2) as compared with 15.8 mg (SD 6.2) in the first quartile. IDP was significantly positively associated with lead intake (Fig. 2).

The mean global cognition score was 12.1 (SD 6.8) in 1997. The prevalence of poor cognition ranged from 19.8 to 23.1% in the four waves of survey between 1997 and 2006. The annual cognitive function score decline was 0.1 (95% CI 0.07, 0.13).

IDP was related to cognitive function decline in a dose-response manner (Table 2). The difference in cognitive function between quartile 4 and quartile 1 of IDP was −1.23 after adjusting for age, gender and energy intake. In the fully adjusted model, across quartiles of IDP, the

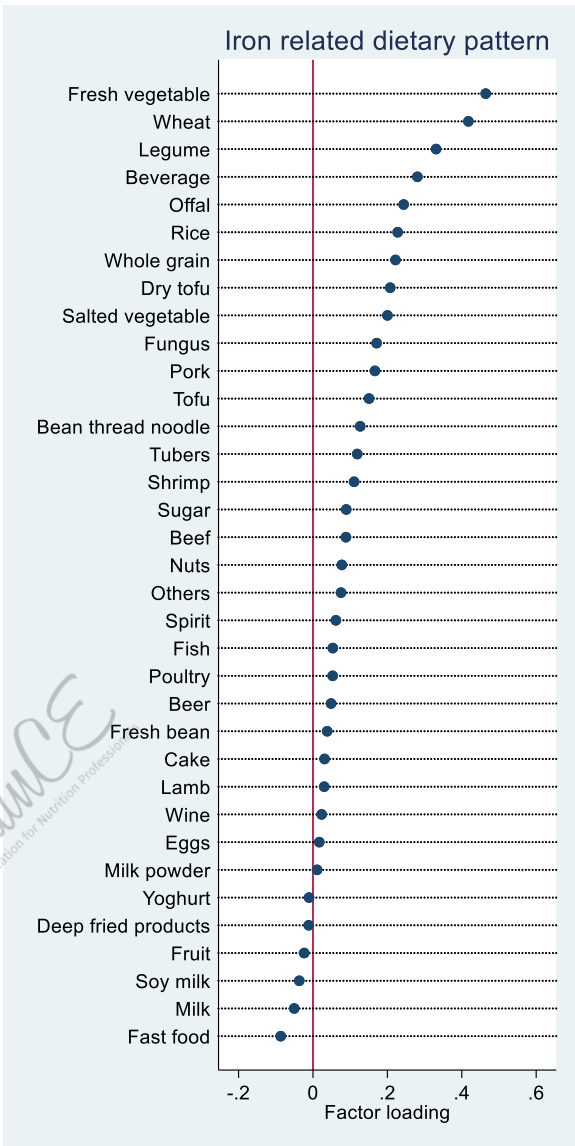

Fig. 1 Factor loadings of iron related dietary pattern based on reduced rank regression

regression coefficients (95% CI) were: 0, −0.11 (−0.50, 0.28), −0.42 (−0.84, 0.00), and −0.79 (−1.25, −0.32), respectively. A similar positive association between IDP and memory decline was observed. The regression coefficients for memory across quartiles of IDP were: 0, −0.15 (−0.43, 0.13), −0.31 (−0.61, −0.02), and −0.37 (−0.70, −0.04), respectively. The associations between IDP and memory decline were attenuated and became statistically not significant after further adjustment of cumulative carbohydrate or iron intake. Similarly, the associations between IDP and memory decline were attenuated after adjustment for lead intake. This suggests the mediating effect of carbohydrates and lead in the relationship between IDP and cognition. Both carbohydrate and lead intake were

Table 1 Sample characteristics of Chinese adults aged ≥55 years old at the first cognitive function test by quartiles of iron related dietary pattern ($N = 4685$)

	Q1	Q2	Q3	Q4	*p*-value
	$N = 1150$	$N = 1128$	$N = 1161$	$N = 1246$	
Energy intake (kcal/d)	1625.8 (440.6)	2028.5 (1057.2)	2196.2 (552.1)	2516.4 (799.3)	< 0.001
Fat intake (g/d)	56.5 (30.3)	71.0 (112.6)	70.7 (36.2)	72.8 (58.6)	< 0.001
Protein intake (g/d)	47.1 (14.6)	60.0 (17.2)	67.1 (20.5)	78.4 (28.5)	< 0.001
Carbohydrate intake (g/d)	229.1 (69.9)	282.3 (80.1)	316.2 (88.9)	377.5 (121.5)	< 0.001
Most recent iron intake (mg/d)	13.8 (7.2)	18.1 (7.3)	21.2 (11.0)	26.8 (15.2)	< 0.001
Cumulative iron intake (mg/d)	15.8 (6.2)	21.2 (6.6)	24.5 (9.4)	31.0 (12.2)	< 0.001
Intake of fruit (g/d)	22.6 (69.4)	23.8 (73.8)	23.9 (83.9)	22.7 (90.2)	0.97
Intake of fresh vegetable (g/d)	183.8 (103.5)	254.4 (132.4)	298.4 (155.6)	352.3 (234.9)	< 0.001
Intake of rice (g/d)	190.9 (116.7)	232.1 (140.5)	252.9 (163.5)	237.7 (207.9)	< 0.001
Intake of wheat (g/d)	84.4 (90.9)	107.9 (110.8)	140.3 (138.3)	227.8 (226.3)	< 0.001
Intake of meat (g/d)	56.0 (58.1)	77.8 (74.4)	80.6 (83.4)	77.1 (102.3)	< 0.001
Age (years)	67.8 (9.0)	63.1 (7.3)	62.0 (6.8)	60.9 (6.1)	< 0.001
Sex					< 0.001
Men	356 (31.0%)	499 (44.2%)	594 (51.2%)	799 (64.1%)	
Women	794 (69.0%)	629 (55.8%)	567 (48.8%)	447 (35.9%)	
Education					0.002
Low	727 (77.5%)	735 (70.7%)	773 (71.8%)	832 (70.7%)	
Medium	105 (11.2%)	158 (15.2%)	163 (15.1%)	202 (17.2%)	
High	106 (11.3%)	146 (14.1%)	141 (13.1%)	142 (12.1%)	
Urbanization					< 0.001
Low	224 (19.5%)	218 (19.3%)	282 (24.3%)	468 (37.6%)	
Medium	274 (23.8%)	325 (28.8%)	368 (31.7%)	352 (28.3%)	
High	652 (56.7%)	585 (51.9%)	511 (44.0%)	426 (34.2%)	
Smoking					< 0.001
Non-smoker	879 (76.8%)	796 (70.7%)	765 (65.9%)	709 (57.0%)	
Ex-smokers	42 (3.7%)	33 (2.9%)	36 (3.1%)	66 (5.3%)	
Current smokers	224 (19.6%)	297 (26.4%)	359 (30.9%)	469 (37.7%)	
Survey year					< 0.001
1997	561 (48.8%)	537 (47.6%)	500 (43.1%)	514 (41.3%)	
2000	210 (18.3%)	171 (15.2%)	186 (16.0%)	207 (16.6%)	
2004	246 (21.4%)	239 (21.2%)	284 (24.5%)	324 (26.0%)	
2006	133 (11.6%)	181 (16.0%)	191 (16.5%)	201 (16.1%)	
Alcohol drinking (yes)	241 (21.4%)	305 (27.6%)	390 (34.0%)	498 (40.7%)	< 0.001
Physical activity (MET, hours/week)	58.1 (75.9)	87.2 (101.0)	91.3 (98.8)	111.9 (109.5)	< 0.001
BMI (kg/m^2)	22.8 (3.8)	23.2 (3.7)	23.1 (3.5)	23.1 (3.4)	0.075
BMI ≥ 24 kg/m^2	367 (34.9%)	408 (39.2%)	412 (37.9%)	407 (35.8%)	0.15
Hypertension (yes)	447 (41.2%)	375 (35.5%)	363 (32.8%)	375 (32.3%)	< 0.001
Diabetes (yes)	45 (4.0%)	36 (3.2%)	29 (2.6%)	39 (3.2%)	0.29
Stroke (yes)	34 (3.0%)	19 (1.7%)	18 (1.6%)	28 (2.3%)	0.082

Data are shown as n (%) or mean ± SD. *p* values were calculated from ANOVA or chi square test

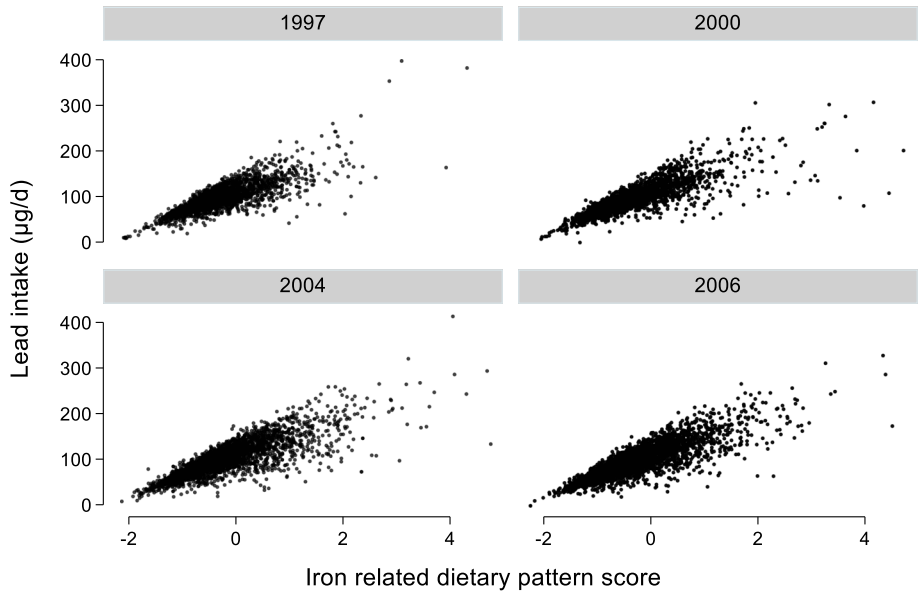

Fig. 2 Association between iron-related dietary pattern and lead intake

Table 2 Regression coefficients (95% CI) for cognitive function by quartiles of iron related dietary pattern among Chinese adults aged ≥55 years old attending China Health and Nutrition Survey ($N = 4852$) between 1997 and 2006

	Dietary pattern quartiles				
	Q1 (low intake)	Q2	Q3	Q4 (high intake)	p for trend
Global cognitive function	Coef. (95% CI)				
Model 1[a]	0.00	−0.06 (−0.42–0.30)	**−0.53 (−0.92--0.15)**	**−1.23 (−1.65--0.81)**	< 0.001
Model 2[b]	0.00	0.00 (− 0.39–0.38)	− 0.32 (− 0.73–0.08)	**− 0.74 (−1.19--0.28)**	< 0.001
Model 3[c]	0.00	− 0.11 (− 0.50–0.28)	− 0.42 (− 0.84–0.00)	**− 0.79 (− 1.25--0.32)**	< 0.001
Model 3+ carbohydrate (quartiles)	0.00	0.15 (− 0.26–0.55)	0.05 (− 0.40–0.50)	− 0.19 (− 0.70–0.32)	0.373
Model 3 + lead (quartiles)	0.00	−0.01 (− 0.45–0.42)	−0.24 (− 0.74–0.27)	−0.57 (− 1.16–0.02)	0.035
Model 3 + iron (quartiles)	0.00	0.05 (−0.37–0.47)	−0.16 (− 0.63–0.31)	−0.41 (− 0.95–0.13)	0.080
Sensitivity analysis [d]	0.00	0.01 (− 0.36–0.38)	−0.30 (− 0.69–0.09)	**−0.52 (− 0.96--0.09)**	0.006
Verbal memory score					
Model 1[a]	0.00	−0.13 (− 0.38–0.13)	**−0.44 (− 0.71--0.17)**	**−0.71 (− 1.00--0.41)**	< 0.001
Model 2[b]	0.00	−0.09 (− 0.37–0.18)	−0.25 (− 0.55–0.04)	**−0.33 (− 0.66--0.01)**	0.026
Model 3[c]	0.00	−0.15 (− 0.43–0.13)	−0.31 (− 0.61--0.02)	**−0.37 (− 0.70--0.04)**	0.020
Model 3+ carbohydrate (quartiles)	0.00	0.01 (−0.29–0.30)	− 0.07 (− 0.39–0.26)	−0.10 (− 0.47–0.26)	0.509
Model 3 + lead (quartiles)	0.00	−0.09 (− 0.41–0.22)	−0.17 (− 0.54–0.19)	−0.19 (− 0.61–0.24)	0.376
Model 3 + iron (quartiles)	0.00	−0.01 (− 0.31–0.29)	−0.09 (− 0.42–0.25)	−0.06 (− 0.45–0.33)	0.688
Sensitivity analysis[d]	0.00	−0.10 (− 0.38–0.17)	−0.23 (− 0.52–0.06)	−0.22 (− 0.55–0.10)	0.131

Regression coefficients and 95% CI were estimated with mixed effect regression models with different levels of adjustment
[a] Model 1 adjusted for age, gender and energy intake
[b] Model 2 further adjusted for intake of fat, smoking, alcohol drinking, income, urbanicity, education, and physical activity
[c] Model 3 further adjusted for BMI and hypertension
[d] Sensitivity analysis model 3 further adjusted for diabetes and stroke after excluding those with a global cognitive function score ≤ 4
All the adjusted variables are treated as time-varying covariates. Bold font represents $p<0.05$

inversely associated with cognitive function (data not shown).

In sensitivity analyses, the above association between IDP and cognition did not change after excluding those with a global cognitive function score below 4 or further adjusting for diabetes or stroke or lead intake.

However, no association between most recent IDP and cognition was found in multivariable mixed model (Additional file 1: Tables S1 and S2).

A borderline significant interaction ($p = 0.085$) between IDP and meat intake in relation to cognitive function was found (Table 3). The positive association between IDP and cognitive function decline was only observed among those with no or low intake of meat. Among those with meat intake < 50 g/d, there was a significant increase of odds ratio (OR) (95% CI) for global cognition score below 7 across quartiles of IDP: 1.00, 1.13 (0.83–1.53), 1.28 (0.93–1.76), and 1.93 (1.36–2.75), respectively. There were no significant interactions with urban-rural residence, overweight/obesity, hypertension, and gender.

When we limited the analyses to those who took the cognitive tests in at least two waves of survey, the findings remained unchanged. Intake of lead was positively associated with global cognition score < 7. However, adjusting for lead intake did not change the association between IDP and cognition.

In fully adjusted models, across the quartiles of IDP intake the OR (95% CI) for global cognition score < 7 were: 1.00, 1.06 (0.86–1.30), 1.24 (0.99–1.54), and 1.50 (1.17–1.93), respectively. The association became statistically insignificant after further adjustment of cumulative carbohydrate intake.

Discussion

In this prospective cohort study of adults aged ≥55 years from CHNS, using RRR method we derived an iron-related dietary pattern characterized by a high intake of fresh vegetable, wheat, legumes, beverage, offal, rice and whole grain. A high intake of this predominantly plant-based IDP was associated with poor cognitive function. Meat consumption modified the association between IDP and cognition. The association between IDP and poor cognitive function was mainly seen among those with a low intake of meat but not those with a high

Table 3 Odds ratio (95% CI) for global cognitive score below 7 across quartiles of iron related dietary pattern among Chinese adults aged ≥55 years old by characteristics, China Health and Nutrition Survey ($N = 4852$) between 1997 and 2006 [a]

	Q1	Q2	Q3	Q4	p for interaction
	Coef. (95% CI)				
Overall sample	1.00	1.06 (0.86–1.30)	1.24 (0.99–1.54)	1.50 (1.17–1.93)	
Overweight/obesity					
No	1.00	1.07 (0.83–1.39)	1.26 (0.96–1.66)	1.54 (1.13–2.10)	0.997
Yes	1.00	1.06 (0.75–1.50)	1.26 (0.87–1.82)	1.53 (1.00–2.34)	
Hypertension					
No	1.00	1.06 (0.82–1.37)	1.21 (0.92–1.59)	1.59 (1.17–2.15)	0.766
Yes	1.00	1.01 (0.73–1.40)	1.27 (0.89–1.80)	1.34 (0.89–2.02)	
Income					
Low	1.00	1.24 (0.89–1.74)	1.24 (0.86–1.78)	1.32 (0.88–1.98)	0.330
Medium	1.00	0.79 (0.55–1.13)	1.03 (0.71–1.49)	1.42 (0.93–2.17)	
High	1.00	1.09 (0.73–1.62)	1.49 (0.96–2.31)	1.84 (1.12–3.02)	
Gender					
Men	1.00	1.27 (0.83–1.95)	1.59 (1.04–2.44)	2.10 (1.35–3.29)	0.497
Women	1.00	1.00 (0.78–1.27)	1.11 (0.85–1.45)	1.24 (0.90–1.70)	
Urbanization					
Low	1.00	1.52 (0.97–2.39)	1.24 (0.78–1.98)	1.94 (1.21–3.11)	0.323
Medium	1.00	0.95 (0.63–1.41)	1.25 (0.82–1.89)	1.29 (0.80–2.08)	
High	1.00	0.94 (0.70–1.25)	1.21 (0.88–1.67)	1.33 (0.89–1.98)	
Meat intake					
< 50 g/d	1.00	1.13 (0.83–1.53)	1.28 (0.93–1.76)	1.93 (1.36–2.75)	0.085
≥ 50 g/d	1.00	1.00 (0.75–1.32)	1.17 (0.86–1.59)	1.05 (0.73–1.52)	

[a] Mixed effect logistic modes adjusted for age, gender, intake of energy and fat, smoking, alcohol drinking, income, urbanicity, education, and physical activity, BMI and hypertension. Stratification variables were not adjusted in the corresponding models
Income was categorized into low, medium and high based on tertiles of year specific income

intake of meat. The dietary pattern was also highly associated with lead, iron and carbohydrate intake. Our study suggests that the association between IDP and cognitive function may be mediated partly by iron, lead and carbohydrate intake in the Chinese population.

This is the first study using RRR method to construct dietary pattern with iron as an intermediate response variable. The dietary pattern approach used in the study helps to understand the complex nature of nutritional epidemiological studies. Our study shows that the single nutrient approach commonly used in the traditional epidemiological research can be confounded by many unmeasured factors. The current study of iron-related dietary pattern further confirms our previous findings on the association between iron intake and cognitive function [17]. The intake of iron in the fourth quartiles of IDP was 31 mg/d which is 2.5 times the recommended iron intake (i.e. 12 mg/d) for Chinese adults. Compared with the relationship between iron intake and poor cognition, the association between IDP and poor cognition showed a clearer dose response positive association. Specifically, across quartiles of iron intake, the OR for poor cognition were 1.00, 1.06 (0.87, 1.30), 1.09 (0.88, 1.35) and 1.30 (1.04, 1.64), respectively. The corresponding figures were 1.00, 1.06 (0.86–1.30), 1.24 (0.99–1.54), and 1.50 (1.17–1.93) across quartiles of iron related dietary pattern.

The focus on the link between iron intake and cognition can be dated back to 1956, when Harman hypothesized that free non-heme iron is a major contributor of neural and cognitive aging [40]. The evidence has been synthesized in several recent reviews showing the important role of iron in neurodegenerative diseases, as a redox-active ion that can cause oxidative stress in the cell [11, 41] Iron intake is related to iron deposits in the brain in animal studies [42], which may increase oxidative stress in the brain. In animal models, iron chelation has been shown to be effective in treating neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [41].

Some components of our IDP (fresh vegetables, legumes and whole grain) were similar with Mediterranean diet. However, our IDP also differ from the Mediterranean diet as it does not have high loadings of fish and nuts, which have showed beneficial role for cognition function [43, 44]. Overall, the beneficial effects of Mediterranean diet on cognition has been well documented [7]. Traditional Chinese diet has similarity to the Mediterranean diet as shown by its high intake of vegetable, whole grains, and vegetable oil. In CHNS, Qin et al found that those with a high adapted Mediterranean diet score were associated with a slower cognitive decline [45]. The main contributors of the observed association were fish, fruits and lower intake of animal-source cooking fats from the aforementioned Mediterranean diet. However, the intake of vegetable, legume, fiber-rich grains showed no benefits on cognition. In Hong Kong, no association between Mediterranean diet and cognition was found [46].

The interaction between IDP and meat consumption is intriguing. The positive association between IDP and poor cognition was only found among those with no or a low meat intake, suggesting the importance of a balanced diet. This finding is also supported by the current knowledge on the importance of protein-rich diet for peoples' health in addition to plant/vegetable consumption. Previous studies have shown the beneficial role of the higher-protein diets, which improve adiposity, blood pressure and triglyceride levels, which are in turn related to cognitive impairment disorders such as Alzheimer's and Parkinson's [47]. There is growing interest in the association between a protein-rich diet and cognition in the epidemiology studies. For example, our findings are consistent with another study using CHNS data. Xu et al found that protein-rich dietary pattern (high intake of milk, eggs and soymilk) was positively but a starch-rich dietary pattern (high intake of salted vegetable, whole grain and legumes) was inversely associated with global cognition in CHNS [48].

High carbohydrate intake was associated with poor cognition in our study and explained the association between IDP and cognition. This finding is supported by several studies. For example, a low glycemic index (GI) breakfast has been shown to be in favour of cognitive performance later in the morning among adults [49]. Low carbohydrate diet has been shown to be beneficial for cognition function among older adults with mild cognitive impairment [19]. Among diabetic patients, a low-GI carbohydrate meal is associated with better cognitive performance than a high GI meal [21].

Food heavy metal contamination (e.g. lead, cadmium and arsenic) has been a greater public concern in China due to environmental pollution [50]. The mean dietary lead intake in the Chinese population was estimated to be 73.9 μg/d [25]. In the current study, IDP was highly correlated with lead intake. Lead intake is a known risk factor for poor cognition. It may explain the association between IDP and poor cognition. The positive association between IDP and cognition may suggest a collective effect of high iron and heavy metals. As IDP was inversely associated with CRP in 2009 (data not shown), the association between IDP is unlikely to be mediated by inflammation.

Our study has several limitations. Wheat contains twice the amount of iron than rice and contributes mainly to the IDP. As there is a large geographic variation on wheat consumption in China, our findings may be confounded by unmeasured factors related to regions. The use of a regional food lead concentration table is another limitation as the contamination level is likely to

be varied by regions. However, the estimation of lead intake only serves the purpose to explore the possible explanation of the link between IDP and cognition. Underreporting of diabetes is another limitation. We were unable to test whether diabetes mediates the association between IDP and cognition. Furthermore, we do not have occupational information, which has been linked to cognitive impairment. Nevertheless, education levels and physical activities were accounted for. The strength of the study is the repeated measure of dietary intake over a long period of time. We are able to adjust for several confounding factors.

Conclusion

Iron related diet may increase the risk of poor cognition. This link is particularly strong for individuals with no or low meat consumption, underscoring the importance of a balanced diet. The link may be partly explained by a high intake of lead, iron and carbohydrate. As the burden of obesity and other non-communicable diseases increase, people are increasingly seeking a plant-based diet to manage body weight. Intake of adequate animal food is needed to prevent cognition decline among those with a high intake of plant-based diet. The role of iron and heavy metal contamination on cognition needs further investigation.

Additional file

Additional file 1: Figure S1. Sample flowchart of participants attending China Health and Nutrition Survey. **Table S1.** Regression coefficients (95% CI) for cognitive function by quartiles of recent iron related dietary pattern among Chinese adults aged ≥55 years old attending China Health and Nutrition Survey ($N = 4852$) between 1997 and 2006. **Table S2.** Odds ratio (95% CI) for global cognitive score below 7 across quartiles of recent iron related dietary pattern among Chinese adults aged ≥55 years old by characteristics, China Health and Nutrition Survey (N = 4852) between 1997 and 2006. (DOCX 50 kb)

Competing interests
The authors declare that they have no competing interests.

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