SUPPLEMENTAL MATERIALS

Methods

Anthropometric measurements.

Body weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Circuits and Systems, Model SX-501, East Rockaway, NY, USA) and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Ayrton Corporation, Model S100, Prior Lake, MN, USA). Overnight-fasted women voided and wore surgical scrubs and no shoes for measurements.

Blood analyses.

Blood from overnight-fasted participants was collected by antecubital venipuncture into EDTA tubes. Hemoglobin was measured using a Cell Dyn 3200 Hematology System (Abbott Diagnostics, Santa Clara, CA, USA). Serum iron was determined colorimetrically using the Hitachi 902 Clinical Chemistry Analyzer (Roche Diagnostics, Basel, Switzerland). Plasma zinc was determined using plasma collected in lithium-heparin Monovettes (Sarstedt Inc., Newton, NC, USA). Plasma was diluted 15-fold with 1N nitric acid (Fisher Trace Metal Grade, Fisher Scientific, Pittsburg, PA), and the resulting supernatant was analyzed for zinc using ICP-AES (Vista AX CCD Simultaneous Inductively Coupled Plasma Atomic Emission Spectrometer with an SPS5 autosampler, Varian, Inc., Walnut Creek, CA, USA). Serum ferritin was determined using a chemiluminescent system (Immulite, DPC, Los Angeles, CA, USA). Serum soluble transferrin receptor was measured using an ELISA kit (Ramco Laboratories, Inc., Houston, TX, USA). A high-sensitivity serum C-reactive protein (CRP) chemiluminescent assay (Immulite,

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DPC, Los Angeles, CA, USA) was used to screen for infection, which can alter measures of iron status ⁽¹⁾. A serum CRP level of 3.0 mg/L was set as the maximum cut-off for normal ⁽²⁾.

Cognitive performance tests.

Bakan task. The stimulus exposure duration was 600 ms with 48 potential correct targets. The dependent variables were the number of correct and incorrect hits over the course of the task.

Mental rotation. The geometric forms comprised the 8- and 12-point shapes used by Cooper ⁽³⁾. The right-hand forms required a 0-, 60-, 120-, 180-, or 300-degree clockwise mental rotation. The task included 96 trials comprised of eight trials of each of the two forms, in each of the rotation positions.

Simple reaction time. A variable stimulus onset of 1, 3, 7, or 16 s, was used in order to prevent participants anticipating the appearing of the star.

Two-finger tapping. The dependent variable was the time taken to make 300 key presses.

Salivary cortisol.

Participants were directed to chew the Salivette cotton swab for one minute and then allow it to rest beneath the tongue for one minute. Participants were instructed to turn on room lighting for the collections and to remain in bed between the first and second collections. Salivettes were stored in the subject's refrigerator until transport to the laboratory on the appointment day. Women kept written records of the time of each home saliva sample collection. Salivettes were centrifuged at 1000 x g for 2 minutes and aliquots of supernatant were frozen at -70° C until analysis. Saliva was screened for blood contamination (which can falsely elevate cortisol values) using a commercially available enzyme immunoassay (EIA) kit for transferrin (Salimetrics, State

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College, PA, USA). Samples with transferrin values greater than 1 mg/dl were considered bloodcontaminated and excluded from final data analysis. Salivary cortisol was measured in duplicate using a high-sensitivity EIA (Salimetrics, State College, PA, USA) with a lower limit of sensitivity of <0.007 ug/dl, and an average intra- and inter-assay coefficient of variation of 5.74% and 6.78%, respectively. The correlation between saliva and serum cortisol for the assay is 0.960 (p<0.0001).

Questionnaires.

The 21-item BDI-II assesses symptoms of depression, with score ranges of 0-13, 14-19, 20-28, and 29-63 indicating minimal, mild, moderate, and severe depression, respectively⁽⁴⁾. The 10item Cohen Perceived Stress Scale measures self-perceived stress using a 5-point Likert-type scale, ranging from never, 0, to very often, 4⁽⁵⁾. Normative Perceived Stress scores for females and people aged 18-29 years are 13.7 ± 6.6 and 14.2 ± 6.2 , respectively. The State-Trait Anxiety Inventory (STAI) consists of separate self-report scales that measure the intensity and frequency of state (at this moment) and trait (general) anxiety⁽⁶⁾. Normative STAI scores for female college students are 38.76 ± 11.95 (state) and 40.4 ± 10.15 (trait); minimum and maximum scores for both scales are 20 (low anxiety) and 80 (high anxiety). The 98 Block food-frequency questionnaire (FFQ) assesses usual dietary intake in an 8-page scannable questionnaire consisting of 110 commonly consumed food items. Completed FFQs were scanned by NutritionQuest and analyzed using a nutrient database developed from NHANES III and Continuing Food Survey of Food Intake by Individuals (CSFII) data and the USDA Database for Standard Reference, Release 12. Because these were non-dieting, sedentary/moderate activity women, FFQs measuring energy intakes of <3767 kJ or >12556 kJ were considered unreliable and excluded from analyses

References

1. Konijn AM (1994) Iron metabolism in inflammation. Baillieres Clin Haematol 7, 829-849.

2. Ford ES, Giles WH, Mokdad AH *et al.* (2004) Distribution and correlates of C-reactive protein concentrations among adult US women. *Clin Chem* **50**, 574-581.

Cooper LA (1975) Mental rotation of random two-dimensional shapes. *Cognit Psychol* 7, 20 43.

4. Beck AT, Steer RABrown GK (1996) *BDI-II Manual*. San Antonio: The Psychological Corporation, Harcourt Brace & Company.

5. Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. *J Health Soc Behav* 24, 385-396.

6. Spielberger CD, Gorsuch RL, Luschene R *et al.* (1983) *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.