Detection of Functional Overreaching in Endurance Athletes Using Proteomics

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There is a strong demand for diagnostic tools to identify athletes in training states.

PURPOSE: To determine if a cluster of proteins could be identified through proteomics procedures that are linked to functional overreaching (FOR) in male endurance athletes.

METHODS: Participants (N=10, age 38 ± 8 yrs, 1.7 m, 74 kg, VO2max 41.4 ± 7.1 ml/kg/min) served as their own controls and in random, counterbalanced order either ran/cycled 2.5 h (70 ± 3.7% VO2max, 79.6 ± 6.3% HRmax) three days in a row (FOR) or sat in the lab (rest) (separated by three weeks) (7:00 - 9:30 am, overnight fasted state). Participants provided fingertip samples for dried blood spot samples (DBS) pre- and post-exercise/rest each of the three days, and then at 7:00 am during two additional recovery days. Participants also completed the Training Distress Scale (TDS) (19-items) at 7:00 am each of the five mornings during each trial (FOR and rest). Proteins were solubilized from DBS, digested into peptides and measured with nanoLC-MS in data independent acquisition mode (Q-Exactive, Thermo Fisher Scientific, Waltham, MA). The RAW MS data files were processed using SpectrumTM software (Bioinformatics, Schlieren, Switzerland). Following data independent acquisition (DIA method), 594 proteins were identified and quantified. Proteins were considered for the FOR cluster if they were elevated during one of the two recovery days but not more than one of the exercise days (compared to rest). The Generalized Estimating Equation (GEE) was used to identify proteins linked to FOR (between trial contrasts; P<0.05 for proteins with CV>15%, P<0.01 with CV>15%).

RESULTS: TDS scores differed between FOR and rest trials, peaking on the first recovery day (9.4±3.8, 3.5±2.6, respectively, P=0.029). A total of 13 proteins was linked to FOR and of these, 11 were related to the immune system, and two to exercise-induced physiological responses. Immune-related proteins included those associated primarily with the acute phase response, complement activation, and granulocyte function.

CONCLUSIONS: This study utilized targeted, DIA proteomics procedures to identify a cluster of 13 proteins linked to FOR (7.5 h of high intensity exercise over three days), and 85% of the proteins were related to immune system activation during the 2-day recovery period.

Changes In Functional Activation Of Memory T Cells Following Exercise: A Pilot Study

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Memory T (Tm) cells function to provide long-lasting protection against re-exposure to pathogens. The recall response of Tm cells to foreign antigen is quicker and of a greater magnitude than a naïve T cell. How functional activation is altered in Tm cells following a bout of exercise is not well known.

PURPOSE: To quantify exercise-induced changes in surface markers of early, middle, and late stage activation in memory T cells (CD4+CD45RO+CD45RA-) obtained from human subjects.

METHODS: Utilizing a cross over design, untrained subjects completed a control and exercise visit. The control visit consisted of 30 min of seated rest while the exercise session entailed 3 sets x 10 reps squat at 70% 1RM, 3x10 leg press at 70% 1RM, and 3x10 leg extensions at 70% 1RM with 2 min between sets. Venous blood samples were obtained pre and post each visit. CD4+ T cell isolation from peripheral blood was conducted through negative selection using a Human CD4+ T cell enrichment kit. CD4+ T cells were plated at 1.5 x 10^6 cells/ml in 200 ml of Immunocult T-cell expansion media directly after isolation and costimulated through CD3+CD28 or no stimulation. Cells were incubated for 1 and 3 days at 37°C in a humidified incubator with 5% CO2, and then analyzed by flow cytometry. Early (CD69), middle (CD25), and late (HLA-DR) markers of activation within the CD45RO+CD45RA- subset were quantified at days 0, 1, and 3. Data were analyzed using two-way RMANOVAs.

RESULTS: There were no significant differences in any markers of activation at the pre measure (p > 0.05). Preliminary data suggests exercise does not alter functional activation in non-stimulated CD45RO+CD45RA- cells. There does appear to be a functional impact related to the Tm cells ability to respond to stimuli post-exercise with two-fold increases observed in HLA-DR expression for cells co-stimulated through CD3+CD28.

CONCLUSIONS: Exercise-induced alterations in functional activation of Tm cells will need to be better quantified to determine not only the magnitude of change, but also to identify a kinetic profile of marker expression. Quantification of changes in this subset of cells will aid in our understanding how immune responses following vaccination are affected by exercise stress.

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Ultra-endurance Triathlon Performance And Markers Of Whole-body And Gut-specific Inflammation

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PURPOSE: To examine the influence of the Ultraman triathlon (3 days of non-continuous racing; stage 1: 10 km swim and 144.8 km cycle; stage 2: 275.4 km cycle; stage 3: 84.4 km run) on circulating plasma concentrations of whole-body (CRP, IL-6, and IL-10) and gut-specific inflammatory markers (IL-17 and IL-23) in trained participants (N = 17; 14 men, 3 women), and determine whether these variables influence performance.

METHODS: Forty-three triathletes (age: 39 ± 8 yrs) were evaluated pre-race and post-race for circulating concentrations of CRP, IL-6, IL-10, IL-17, and IL-23. Blood samples were drawn two days prior to stage 1 (1600 h) and one day after stage 3 (1200 h). Plasma biomarker concentrations were determined by ELISA according to manufacturer’s instructions. Data were analyzed with SPSS and significance was accepted at p ≤ 0.05. Values are reported as means ± SD. Data points (for blood biomarkers) greater than 2 SD from the mean were removed as outliers.

RESULTS: Plasma CRP significantly increased from pre-race (266.27 ± 276.18 mg/ml) to post-race (25,891.94 ± 12,888.65 mg/ml; p < 0.001). Plasma IL-10 increased from pre-race (3.46 ± 2.98 pg/ml) to post-race (5.15 ± 4.89 pg/ml). Pre-race concentrations of IL-6 were below detectable limits; post-race IL-6 concentrations were 4.00 ± 3.74 pg/ml. Both pre-race and post-race concentrations of IL-17 and IL-23 were below detectable limits. Pearson’s correlation between mean finish time and post-race CRP and post-race IL-10 was 0.35 and 0.54 (p < 0.05), respectively.

CONCLUSIONS: The significant increase in CRP during the race may have been due to muscle damage. The greater anti-inflammatory capacity of the athletes likely led to increased clearance of IL-6, IL-17, and IL-23 the day after the race; the increase in IL-10 concentrations during the race reflect this anti-inflammatory response. A significant positive correlation between post-race IL-10 concentrations and mean finish time may indicate that a relationship between anti-inflammatory responses and performance exists. This study was supported by Florida State University.

Development Of A Consumer-Oriented Microbiome Tracker

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BACKGROUND: Interest in and knowledge of the gut microbiome has increased exponentially in the past decade. This once overlooked component of the gastrointestinal tract is now implicated in multiple aspects of human health, including mental wellness (e.g. depression, anxiety, stress), metabolic (e.g. diabetes, obesity), neurologic (e.g. Alzheimer’s, autism), gastrointestinal (e.g. irritable bowel syndrome, Crohn’s), and immunologic (e.g. inflammation, cancer), among others.