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Translational

Immune-metabolic profiling of anorexic patients reveals an anti-oxidant and anti-inflammatory phenotype☆

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ABSTRACT

Context. Anorexia nervosa (AN) is an excessive form of calorie restriction (CR) associated with pathological weight loss and alterations of the immune system. However, AN patients seem to be protected from common viral infections.

Objectives. To investigate the metabolic and molecular adaptations induced by sustained extreme CR in the peripheral blood mononuclear cells (PBMCs) of patients with restrictive alimentary AN.

Design. Inflammatory cytokines and adipokines were measured in 15 young (age range, 15–24 years) AN female patients and 20 age-matched healthy controls. Isolated PBMCs were immunophenotyped by flow cytometry, and glycolysis and mitochondrial respiration were determined by measuring the extracellular acidification and oxygen consumption rate. Stress resistance to H_2O_2 and the antioxidant transcriptional profile of PBMCs and human fibroblasts incubated with sera from AN patients were also determined.

Abbreviations: AN, anorexia nervosa; CR, calorie restriction; NK, natural killer; BMI, body mass index; PBMCs, peripheral blood mononuclear cells; ECAR, extracellular acidification rate; OCR, oxygen consumption rate.

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Results. Compared with controls, AN patients (BMI, 15.9 ± 0.4 kg/m^2) had significantly fewer leucocytes, lymphocytes and NK cells, lower serum concentrations of leptin, IGF-1 and sTNFR1, and higher levels of adiponectin, sCD40L and sICAM-1 ($p < 0.05$). IL-1β, TNFα, and IL-6 produced by PBMC cultured with autologous serum for 48 h were significantly lower in AN patients than in controls ($p < 0.01$). Moreover, glycolysis and mitochondrial respiration were lower, and the antioxidant transcriptional profile was higher in the PBMCs of AN patients. Fibroblasts cultured in serum from AN patients showed a 24% increase in resistance to H_2O_2 damage.

Conclusions. Extreme CR in AN patients is associated with a reduction in several immune cell populations, but with higher antioxidant potential, stress resistance and an anti-inflammatory status.

1. Introduction

Anorexia nervosa (AN) is an excessive form of calorie restriction (CR) caused by restrictive alimentary or binge/purge behavior that leads to pathological weight loss. Extreme CR and low body fat stores in these individuals are associated with alterations of the immune system [1]. In particular, AN patients frequently have leukopenia and relative lymphocytosis, but interestingly they seem to be protected against some infectious disease (e.g. common viral infections and other nosocomial infective disease) [2].

Lymphocytes and natural killer (NK) cells play a central role in regulating the immune response against pathogens. Several adipokines (i.e. leptin and adiponectin) produced by the adipose tissue and some nutrients (e.g. vitamins and trace elements) play a key role in preserving the homeostasis of T-cells and NK cells [3–9]. However, little is known on the metabolic, molecular and functional adaptations induced by extreme and sustained CR in young AN patients on lymphocytes and NK cells. To the best of our knowledge, the only few studies conducted so far on lymphocyte populations in AN subjects were inconclusive, probably due to heterogeneity of the study subjects [10,11].

The aim of this study was to extensively characterize the immune-metabolic profile of AN patients to identify cell types or metabolic aspects that could justify the relatively minor immune impairment observed in these patients despite their nutritional status.

2. Material and methods

2.1. Study subjects

Fifteen women who met DSM-V criteria for AN (age range: 15–24 years) and 20 normal weight female controls (age range: 18–26 years) were enrolled in this study [12]. Subjects were admitted to the outpatient clinic for Eating Disorders of Federico II University Hospital in Naples in the morning after a 12-h fast. No patient had been affected by infectious diseases or required medical treatment or hospitalization during the previous 5 years. All had undergone standard vaccination programs during childhood. None of the parents had experienced a major eating disorder. The research was performed according to the Helsinki II declaration and all enrolled subjects gave informed consent to the study that was approved by the Ethics Committee of our University.

2.2. Study protocol

Height was measured without shoes to the nearest 0.1 cm. Body weight was obtained on a balance scale in the morning. Body mass index (BMI) was calculated by dividing body weight by the square of height (kg/m^2). A venous blood sample was obtained from each participant and analyzed by routine assays for complete blood count as well as a comprehensive metabolic panel to assess current metabolic, kidney and liver function and electrolyte balance. Additional blood samples were stored at –80 °C, and later assayed in batch to measure levels of inflammatory and anti-inflammatory cytokines, adipokines and hormones. Specifically, soluble CD40L (sCD40L), soluble intracellular adhesion molecule (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), myeloperoxidase (MPO), resistin and soluble tumour necrosis factor receptor (sTNFR) were analyzed using the bead-based analyte detection system Human Obesity 9plex kit (Bender Med System, Burlingame, CA) in duplicate serum samples. Leptin, adiponectin, IGF-1 and IGFBP-3 were measured with a highly sensitive ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol. Total catecholamines were determined by HPLC (Hewlett Packard 1100, BioRad, Herts, UK) in 24 h urine.

2.3. Immunophenotyping

Immunophenotyping was performed on a whole blood sample from 10 AN patients and 10 controls with an EPICS XL flow cytometer (Beckman Coulter, Milan, Italy) using the Beckman Coulter XL System II software program. Cell populations were identified using triple combinations of human monoclonal antibodies, namely, fluorescein isothiocyanate [FITC]- and phycoerythrin [PE]-anti-CD3, PE- and PC5-anti-CD4, PC5-anti-CD8, PE-anti-CD16, PC5-anti-CD19, PE-anti-CD25, FITC-anti-CD45, and PE-anti-CD56, all from Coulter Immunotech (Marseille, France).

2.4. Cell cultures, proliferation assays and cytokine measurement

Human peripheral blood mononuclear cells (PBMCs) were isolated by stratifying 15 mL of whole blood on 5 mL of Ficoll-Paque PREMIUM (GE Healthcare) and centrifuging the solution