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Two opposite extremes of adiposity similarly reduce inflammatory response of antigen-induced acute joint inflammation

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ABSTRACT

Objective: Acute inflammation is a normal response of tissue to an injury. During this process, inflammatory mediators are produced and metabolic alterations occur. Adipose tissue is metabolically activated, and upon food consumption, it disrupts the inflammatory response. However, little is known about the acute inflammatory response in joints that results from diet-induced adipose tissue remodeling. The objective of this study was to determine whether alterations in adipose tissue mass arising from food consumption modify the inflammatory response of antigen-induced joint inflammation in mice.
Methods: Male BALB/c mice were fed a chow diet, a highly refined carbohydrate-containing (HC) diet for 8 wk. They were then immunized and, after 2 wk, received a knee injection of methylated bovine serum albumin (mBSA). They were sacrificed at 6, 24, and 48 h after injection. The effect of the cafeteria diet for 8 wk, which also increases adipose tissue, or conjugated linoleic acid (CLA) supplementation for 4 wk, a model of lipodystrophy, was evaluated 24 h after knee challenge with mBSA.
Results: Cellular influx, predominantly neutrophils, in synovial fluid was attenuated in the HC diet group, as were levels of myeloperoxidase and IL-1β in periarticular tissue and histopathological analysis. These responses were associated with reduced adiponectin and increased leptin in serum, which was pronounced in mice fed the HC diet. Cafeteria diet and CLA supplementation induced a profile similar to that seen with the HC diet in terms of inflammation, disease response, and metabolic alteration. Interestingly, after the injection of mBSA, the area of adipocytes in the infrapatellar fat pad increased in mice fed with chow diet similar to those fed the HC and cafeteria diet.
Conclusions: We demonstrated that attenuation of joint response induced by diet was independent of adipose tissue remodeling but could be associated with metabolic alterations.

Introduction

Inflammation is a biological reaction of the tissue to harmful stimuli characterized by the activation of immune system components [1]; when inflammation is persistent, it can lead to

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tissue damage [2]. Importantly, metabolic support and the redistribution of energy are required during an inflammatory response [3]. In particular, there is a rapid mobilization of lipid stocks from specific metabolic organs; this is essential to cellular activation and tissue response [4]. Indeed, nutrients play an important role in supporting the inflammatory process; also, nutrients themselves can trigger inflammation [5,6]. Therefore, changes in the composition of diet can influence the quality of the inflammatory response.

Excessive dietary intake of lipids [5] and carbohydrates [6] can lead to the accumulation of body fat. The main tissue capable of dealing with the resulting excess of energy is adipose tissue, which is metabolically active and capable of remodeling (increasing or decreasing in size) [7,8]. Its expansion, as observed in obesity, results in the recruitment of immune cells to this tissue [9] and contributes to rising levels of serum markers of inflammation [10]. On the other hand, systemic inflammation can also alter the optimal functioning of adipose tissue by increasing lipolysis and leading to insulin resistance [11]. Interestingly, we have shown that the induction of local acute joint inflammation in mice leads to acute alterations in systemic metabolic parameters associated with higher neutrophil influx in the adipose tissue [12]. Further, we hypothesize that any alteration in adipose mass or function could modulate the acute joint inflammatory response in mice.

In this study, we evaluated the effect of diet-induced adipose tissue remodeling on acute arthritic response by using different diet compositions in a model of acute antigen-induced arthritis (AIA) in mice. We demonstrated that the knee inflammatory response is attenuated in distinct models of adipose remodeling (i.e., obesity induced by diet or lipodystrophy).

Materials and methods

Animals and diets

Male BALB/c mice (6 to 7 wk old) were obtained from the animal care center at Universidade Federal de Minas Gerais. They were maintained in an environmentally controlled room under a 12/12-hour light-dark cycle. Animals had free access to tap water and food. The experimental protocol was approved by the Ethics Committee in Animal Experimentation at Universidade Federal de Minas Gerais (protocol: 148/2012).

Mice were fed a standard chow (LABINA) diet, a highly refined carbohydrate-containing (HC) diet, or a cafeteria (CAF) diet for 8 wk. They were immunized with antigen at week 6 and given an injection of antigen at the knee at week 8. The composition of chow diet was 4.0 kcal/g, 65.8% carbohydrate, 3.1% fat, and 31.1% protein. The composition of the HC diet was 4.4 kcal/g, 74.2% carbohydrate (at least 30% refined sugars), 5.8% fat, and 20% protein [5]. The composition of CAF diet was potato chips, chocolate, cookies, peanuts, condensed milk, butter, and soft drinks in addition to standard chow diet. Its nutritional composition was calculated according to the product manufacturers ($\cong$ 5.8 kcal/g, 31% carbohydrate, 58% fat, and 11% protein). In addition, a diet supplemented with conjugated linoleic acid (CLA; 1% w/w of the diet) was used (General Nutrition, Pittsburgh, PA) to induce a phenotype similar to that seen in lipodystrophy [13]. The composition of the CLA-supplemented diet was 4.2 kcal/g, 71.6% carbohydrate, 3.5% fat, and 24.9% protein. The majority of its composition was a 50:50 mixture of 2 isomers, cis-9, trans-11 and trans-10, cis-12. Mice were fed the chow diet for 4 wk and then a CLA-supplemented diet until week 8. They were immunized at week 6 and received the knee challenge at week 8.

Mice were weighed once a week, and the food intake was measured every 2 to 3 d. After each experimental procedure, the animals were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and sacrificed. Samples of blood were collected, as were samples of tissue from the knee. Epididymal, retroperitoneal, and mesenteric white adipose tissues were collected, weighed, and used to determine the adiposity index (i.e., the sum of epididymal, retroperitoneal, and mesenteric tissue in terms of weight in grams: [g] $\div$ body weight $\times$ 100).

Arthritis induction and determination of articular inflammation

Mice were immunized intradermally with 500 μg of methylated bovine serum albumin (mBSA) in 100 μL of a saline emulsion and an equal volume of complete Freund's adjuvant (CFA). Fourteen days after immunization, they were challenged with an intra-articular injection in the knee joint (10 μg mBSA in 10 μL phosphate-buffered saline [PBS]) [14].

To determine the presence of articular inflammation, the knee cavity was washed with PBS (2 $\times$ 5 μL) for cell recovery. The total number of leukocytes was determined by staining them with Turk's solution and then counting them. Differential counts were obtained from cytospin preparations stained with May-Grünwald-Giemsa stain.

Hypernociceptive assessment

Hypernociception was assessed as previously described [15]. Briefly, mice were placed in acrylic cages with a wire grid floor. A series of stimuli were administered with an electronic pressure meter (INSIGHT Instruments, Ribeirão Preto, São Paulo, Brazil) in the hind paw to induce flexion of the knee joint, followed by withdrawal of the paw. The intensity of the pressure was recorded. The response value was obtained by averaging of 2 measurements for each animal. The intensity of hypernociception is reported as paw withdrawal threshold (in grams); that is, the faster withdrawal represents less force tolerated, indicating higher hypernociception.

Histopathological analysis

Knee joints were fixed in formalin, decalcified (EDTA 14%), and embedded in paraffin. Sections (measuring 7 mm) were stained with hematoxylin and eosin. Two sections/knee joints of each animal were examined and scored in a blind manner as follows: severity of synovial hyperplasia (0–3), intensity and extension of inflammatory infiltrate (0–4), and bone erosion (0–2). The grades were summed to obtain the arthritis index (0–9).

For the analysis of infrapatellar adipocytes, images of 6 fields from each animal were captured using a microscope ($\times$100) coupled to a camera. The cell area was measured in each animal by using ImageJ software (National Institutes of Health, Bethesda, MD). The number of infrapatellar adipocytes was counted in an area of 200 μm^2.

Myeloperoxidase activity

Indirect neutrophil presence in the knee was measured by assaying myeloperoxidase activity, as described previously [16]. Briefly, the periarticular tissue (knee joint) was homogenized and assayed for myeloperoxidase activity by measuring the change in OD (450 nm) using tetramethylbenzidine as substrate.

ELISA assay

IL-1β was determined in the periarticular tissue; adipocytokines, adiponectin, resistin, and leptin were analyzed in the periarticular tissue and serum using DuoSet ELISA development kits (R&D System, Inc., Minneapolis, MN) according to the manufacturer's instructions.

Lysate preparation and Western blot analysis

Inflammatory cells harvested from the knee cavity were washed with PBS and whole cell extracts were obtained by using a lysis buffer (1% Triton X-100, 100 mM Tris/HCl, pH 8.0, 10% glycerol, 5 mM EDTA, 200 mM NaCl, 1 mM DTT, 1 mM PMSF, 25 mM NaF, 2.5 μg/mL leupeptin, 5 μg/mL aprotinin, and 1 mM sodium orthovanadate). Lysates were centrifuged and quantified using the Bradford assay reagent from Bio-Rad (Hercules, CA). Extracts (20 μg) were separated by electrophoresis on a denaturing 10% SDS-PAGE and transferred onto nitrocellulose membranes as described [17]. Membranes were blocked overnight with 5% (w/v) nonfat dry milk and then incubated with primary antibodies from Cell Signaling Technology (Beverly, MA) using a dilution of 1:1000. After washing, membranes were incubated with horseradish peroxidase–conjugated secondary antibody (1:3000). Immunoreactive bands were visualized by using the ECL detection system (GE Healthcare, Piscataway, NJ).

Statistical analysis

Results were expressed as mean $\pm$ SEM. Multiple comparisons were performed using one-way analysis of variance. However, the post hoc tests differed according to the experimental protocol. In the kinetic experiment, comparisons of the same diet given at different times were used with Dunnett's post hoc test; testing was also done at the same time point to compare different diets (the chow and HC diets) using Student's *t* test. For experiments with 4 different diet compositions, the Newman-Keuls post hoc test was used. Two-way analysis of variance was performed to arrive at body weight gain data, followed by the Bonferroni post hoc test. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA). Statistical significance was set at $P < 0.05$.