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Deregulation of arginase induces bone complications in high-fat/high-sucrose diet diabetic mouse model

Anil Bhatta [b, 1], Rajnikumar Sangani [a, 1], Ravindra Kolhe [c], Haroldo A. Toque [b], Michael Cain [a], Abby Wong [a], Nicole Howie [d], Rahul Shinde [c], Mohammed Elsalanty [d], Lin Yao [b], Norman Chutkan [e], Monty Hunter [a], Ruth B. Caldwell [f], Carlos Isales [a, g], R. William Caldwell [b, **], Sadanand Fulzele [a, g, *]

[a] Departments of Orthopaedic Surgery, Georgia Regents University, Augusta, GA 30912, USA
[b] Department of Pharmacology and Toxicology, Georgia Regents University, Augusta, GA 30912, USA
[c] Departments of Pathology, Georgia Regents University, Augusta, GA 30912, USA
[d] School of Dentistry, Georgia Regents University, Augusta, GA 30912, Augusta, GA 30912, USA
[e] The CORE Institute, Phoenix, AZ 85023, USA
[f] Cell Biology and Anatomy and Vascular Biology Center, Georgia Regents University; Charlie Norwood VA Medical Center, Augusta, GA 30912, USA
[g] Institute of Regenerative and Reparative Medicine, Georgia Regents University, Augusta, GA 30912, USA

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ABSTRACT

A balanced diet is crucial for healthy development and prevention of musculoskeletal related diseases. Diets high in fat content are known to cause obesity, diabetes and a number of other disease states. Our group and others have previously reported that activity of the urea cycle enzyme arginase is involved in diabetes-induced dysregulation of vascular function due to decreases in nitric oxide formation. We hypothesized that diabetes may also elevate arginase activity in bone and bone marrow, which could lead to bone-related complications. To test this we determined the effects of diabetes on expression and activity of arginase, in bone and bone marrow stromal cells (BMSCs). We demonstrated that arginase 1 is abundantly present in the bone and BMSCs. We also demonstrated that arginase activity and expression in bone and bone marrow is up-regulated in models of diabetes induced by HFHS diet and streptozotocin (STZ). HFHS diet down-regulated expression of healthy bone metabolism markers (BMP2, COL-1, ALP, and RUNX2) and reduced bone mineral density, bone volume and trabecular thickness. However, treatment with an arginase inhibitor (ABH) prevented these bone-related complications of diabetes. In-vitro study of BMSCs showed that high glucose treatment increased arginase activity and decreased nitric oxide production. These effects were reversed by treatment with an arginase inhibitor (ABH). Our study provides evidence that deregulation of L-arginine metabolism plays a vital role in HFHS diet-induced diabetic complications and that these complications can be prevented by treatment with arginase inhibitors. The modulation of L-arginine metabolism in disease could offer a novel therapeutic approach for osteoporosis and other musculoskeletal related diseases.

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1. Introduction

The worldwide increase in the prevalence and incidence of obesity in the last few decades has become a major public health concern (Capehorn, 2013; Zamosky, 2013). Obesity is associated with many serious chronic diseases, including diabetes, cardiovascular disease, hypertension, stroke and some forms of cancers (Daniels et al., 2005; Li et al., 2009a; Joost, 2014; Cao, 2011; Ramos-Nino, 2013). There are also reports that obesity has a considerable effect on musculoskeletal related conditions such as osteoarthritis,

* Corresponding author. Department of Orthopaedic Surgery Georgia Regents University, Augusta, GA 30904, USA.
** Corresponding author. Department of Pharmacology and Toxicology Georgia Regents University, Augusta, GA 30904, USA.
E-mail addresses: wcaldwel@gru.edu (R.W. Caldwell), sfulzele@gru.edu (S. Fulzele).
[1] Equal contribution.

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osteoporosis and increased fracture rate (Villareal et al., 2005; Kessler et al., 2013; Prieto-Alhambra et al., 2012; Tanaka et al., 2013; Fehrendt et al., 2014). Osteoporosis is a metabolic bone disease characterized by reduction in bone mass, leading to weaker bones that have an increased risk of fracture (Tanaka et al., 2013; Chen et al., 2008). The role of obesity in the development of osteoporosis is controversial (Cao, 2011). Previously, obesity has been considered to have a positive impact on bone formation because of the beneficial effect of mechanical loading, exerted by high body mass, but recent studies suggest obesity is a considerable risk factor for osteoporosis (Cao, 2011; Villareal et al., 2005; Colaianni et al., 2014; Fernandes-Santos et al., 2009; Gonnelli et al., 2014).

A chronic high fat high sucrose (HFHS) diet is a well-established method to promote obesity and insulin resistance, resulting in a type 2 diabetes-like condition (hyperglycemia) in animal models (Fernandes-Santos et al., 2009; Lee et al., 2013; Lu et al., 2014; Sampath and Karundevi, 2014). The hyperglycemic condition increases oxidative stress, which is associated with a high risk of diabetic complications in various tissue types (Ramos-Nino, 2013; Lee et al., 2013). Osteoporosis also has been linked to obesity and hyperglycemia (Villareal et al., 2005; Cao, 2011; Tanaka et al., 2013; Chen et al., 2008). We previously reported that STZ-induced diabetic mouse bones have low bone mineral density and are osteoporotic in nature and that diabetic bone and bone marrow have redox reaction imbalances (Sangani et al., 2013). Interestingly, our group and others also found an association between dysregulation of the redox homeostasis, excessive arginase activity and diabetic complications in retinal, vascular and erectile tissues of STZ and Akita mouse models (Steppan et al., 2013; Romero et al., 2008a; Yao et al., 2014; Toque et al., 2013; Narayanan et al., 2013; Romero et al., 2012).

Arginase is an enzyme that plays an important role in L-arginine metabolism and the urea cycle (Romero et al., 2012; Chandra et al., 2012). L-arginine is the common substrate for arginase and nitric oxide synthase (NOS) (Munder, 2009; Tenu et al., 1999; Singh et al., 2000; Durante et al., 2007). Arginase hydrolyzes L-arginine to L-ornithine and urea whereas NOS utilizes L-arginine to generate NO and L-citrulline (Narayanan et al., 2013; Romero et al., 2012; Durante et al., 2007). Elevated arginase activity limits the bioavailability of L-arginine which alters NOS activity causing it to produce more superoxide and less NO (Ogonowski et al., 2000; Kaesemeyer et al., 2000). In short, excessive arginase activity affects redox balance and normal cell metabolism. Little is currently known about the role of arginase in diabetes-related bone metabolism. In this study, we determined the effect of diabetes and arginase activity on bone and bone marrow metabolism. We also used an arginase inhibitor (ABH) to determine if arginase is involved in diabetes and obesity-induced bone complications. This study revealed a link between obesity, arginase and bone complications.

2. Material and methods

2.1. Animal preparation and experimental design

All animal protocols were approved by the Institutional Animal Care and Use Committee at Georgia Regents University. Male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) were obtained at 8 weeks of age. Animals were housed in 12-h light/dark cycle and had free access to food and water throughout the study. To induce obesity and type 2 diabetes, mice were fed on high fat/high sucrose (HFHS diet % of calories = 59% fat, 15% protein, 26% carbohydrate [mostly sucrose], F#1850, BioServe, USA) for 28 weeks. Control mice were fed a normal diet (ND, % of calorie = 18% fat, 24% protein, 58% carbohydrate, Harlan, USA) over the same period. Treatment with the arginase inhibitor, 2-(S)-amino-6-boronohexanoic acid (ABH, 10 mg/kg/day in drinking water) was started after one month and was continued until the end of the study. Body weight and blood glucose levels were measured every two weeks until the animals were sacrificed. After 28 weeks the mice were euthanized for the collection of serum, tibia and femurs. To model type 1 diabetes, STZ induced-diabetes animal studies were performed as per our published methods (Sangani et al., 2013). In brief, animals were given intraperitoneal (IP) injections of vehicle or freshly prepared streptozotocin in 0.01 mol/L sodium citrate buffer, pH 4.5 (45 mg/kg) after a 4-h fast each day for 5 consecutive days. Diabetes was confirmed by fasting blood glucose levels of 250 mg/dL. Four and eight weeks after the establishment of diabetes, the mice were euthanized for the collection of serum, tibia and femurs.

The tibia and femurs were excised carefully and all the soft tissues were removed from the bones. RNA and protein analysis were performed on tibia samples while micro CT was conducted on femur samples. The epiphyses of the tibia were removed and the marrow was flushed out with phosphate-buffered saline (PBS). The diaphyses were cleaned twice with PBS and then snap-frozen in liquid nitrogen and stored at −80 °C. For bone marrow cell isolation, the marrow was flushed with PBS and the cellular material harvested. The cellular material was then centrifuged, the supernatant was discarded, and the pellet was washed with PBS. The cellular material was used for western blot as per our published protocol (Sangani et al., 2013).

2.2. Isolation of BMSCs from mice

Murine BMSCs were isolated from the long bones of C57BL/6 mice as previously described (Fulzele et al., 2013). Briefly, the marrow was flushed with PBS and the cellular material harvested. The cells were plated in 100-cm^2 culture plates with DMEM, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/mL penicillin/streptomycin, and 2 mM L-glutamine. After 24 h, the supernatant was removed, and the adherent stromal cells were trypsinized for negative selection. A negative selection process was used to deplete hematopoietic cell lineages (T- and B-lymphocytic, myeloid and erythroid cells) using a commercially available kit (BD biosciences), thus retaining the progenitor (stem) cell population. The positive fractions were collected using the following parameters: negative for CD3e (CD3 ε chain), CD11b (integrin αM chain), CD45R/B220, Ly-6G and Ly-6C (Gr-1), and TER-119/Erythroid Cells (Ly-76). Positive selections were performed using the anti-Stem cell antigen-1 (Sca-1) column magnetic bead sorting kit (Miltenyi Biotec, CA, USA).

2.3. Arginase activity assay

Plasma and bone marrow homogenates or BMSC lysates in Tris buffer (50 mmol·L−1 Tris−HCl, 0.1 mmol·L−1 EDTA and EGTA, pH 7.5 containing protease inhibitors) were used for arginase activity assay as previously described (Romero et al., 2008b). Briefly, 25uL of 10 mM MnCl2 were added to 25uL of homogenates (cell or tissue) and heated at 57 °C for 10 min to activate arginase. Next, 50uL of 0.5 M L-arginine was then added to the reaction tube and incubated in 37 °C for 1 h and 400uL of acid mixture (H2SO4: H3PO4: H2O in a ratio of 1:3:7) was added to stop the reaction. Then, 25 uL of 9% α-isonitrosopropiophenone (in ethanol) was added and the mixture was heated for 45 min at 100 °C and placed in dark for 10 min to develop color. Arginase activity was measured by loading 200 uL of the reaction mixture in a 96-well plate and absorbance was read at 540 nm.