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Effect of insulin-resistance on circulating and adipose tissue MMP-2 and MMP-9 activity in rats fed a sucrose-rich diet

V. Miksztowicz [a], C. Morales [b], V. Zago [a], S. Friedman [c], L. Schreier [a], G. Berg [a,*]

[a] Laboratory of Lipids and Lipoproteins, Department of Clinical Biochemistry, Faculty of Pharmacy and Biochemistry, INFIBIOC, University of Buenos Aires, Buenos Aires, Argentina
[b] Institute of Cardiovascular Physiopathology and Department of Pathology, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina
[c] Oral and General Biochemistry Department, Faculty of Dentistry, University of Buenos Aires, Buenos Aires, Argentina

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Abstract *Background and aim:* Adipose tissue produces different metalloproteinases (MMPs), involved in adipogenesis and angiogenesis. Different studies have shown that in obesity the behavior of different MMPs may be altered. However there are scarce data about the effect of insulin-resistance (IR) on MMP-2 and MMP-9 activity in adipose tissue. Our aim was to determine whether sucrose induced IR modifies MMP-2 and MMP-9 behavior in expanded visceral adipose tissue and the contribution of this tissue to circulating activity of these gelatinases.
Methods and results: Male Wistar rats were fed with standard diet (Control) or standard diet plus 30% sucrose in the drinking water throughout 12 weeks (SRD). In epididymal adipose tissue vascular density, size and adipocyte density, PPARγ expression and MMP-2 and -9 were measured. Adipose tissue from SRD presented higher adipocyte size (6.32 ± 8.71 vs $4.33 \pm 2.17 \times 10^3$ μm^2, $p = 0.001$) lower adipocyte density (164 (130–173) vs 190 (170–225) number/mm^2, $p = 0.046$) and lower vascular density (16.2 (12.8–23.5) vs 28.1 (22.3–46.5) blood vessels/mm^2, $p = 0.002$) than Control. MMP-2 and MMP-9 activity was decreased in SRD (1.93 ± 0.7 vs 3.92 ± 0.9 relative units, $p = 0.048$ and 1.80 ± 0.8 vs 5.13 ± 1.7 relative units, $p = 0.004$ respectively) in accordance with lower protein expression (0.35 ± 0.20 vs 2.71 ± 0.48 relative units, $p = 0.004$ and 1.12 ± 0.21 vs 1.52 ± 0.05 relative units, $p = 0.036$ respectively). There were no differences in PPARγ expression between groups.
Conclusion: Insulin resistance induced by SRD decreases MMP-2 and MMP-9 activity in adipose tissue which would not represent an important source for circulating MMP-2 and -9. In this state of IR, PPARγ would not be involved in the negative regulation of adipose tissue gelatinases.

Introduction

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases able to degrade extracellular matrix (ECM) components [1]. These enzymes are synthesized by multiple vascular cell types, including endothelial cells, vascular smooth muscles cells, circulatory monocyte, as well as the local tissue macrophages. MMPs play an important role during physiological tissue remodeling in embryonic development [2], in bone resorption [3] and in angiogenesis [4]. Moreover, during the last decade, MMPs have been extensively studied in the pathogenesis of the atherosclerosis process and cardiovascular disease (CVD) because of their major significance in vascular remodeling. Different MMPs have been identified in atherosclerotic plaques and in regions of foam cell accumulation and have been directly associated with plaque remodeling as well as plaque vulnerability [5–7].

* Corresponding author. Junín 956, CABA, Argentina. Tel.: +54 11 4964 8297; fax: +54 11 5950 8692.
E-mail address: gaberg@ffyb.uba.ar (G. Berg).

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Gelatinases (MMP-2 and MMP-9) in general are highly expressed in fatty streaks and atherosclerotic plaques compared to normal regions of the vessel [8,9].

Abdominal obesity is one of the main risk factor of CVD. Expansion of fat cell size would require a pliant ECM, and recent studies have suggested that the absence of such pliant matrix could lead to adipose tissue inflammation, characteristic of insulin resistance (IR) states [10]. In this process, MMPs are involved in the control of proteolysis and adipogenesis [11]. In adipose tissue of an animal model of obesity, induced by high fat diet, it has been reported an up-regulation of mRNA levels of some MMPs (MMP-3, MMP-11, MMP-12, MMP-13, and MMP-14) and down-regulation of others (MMP-7, MMP-9, MMP-16, and MMP-24) as well as of their inhibitors [12]. These modulations differed according to the origin of the adipose tissue (gonadal vs subcutaneous), supporting the concept that the different localization of fat deposits presents different metabolic behavior [13]. In contrast, in a genetic obesity model, other authors described up-regulation of mRNA MMP-2 transcription without changes in mRNA MMP-9 transcription, in parallel with no changes in MMP-2 and MMP-9 activity [14].

Previous studies have demonstrated that rats fed sucrose-rich diet (SRD) exhibited impaired insulin activity and expanded adipose tissue [15,16]. However, scarce studies have been developed to evaluate the effect of sucrose induced IR on MMPs behavior. Until now, to our knowledge, the effect of SRD has only been evaluated on MMPs cardiac expression [17], resulting in increased MMP-2 and MMP-9 levels [18].

Our aim was to determine whether sucrose induced IR modifies MMP-2 and MMP-9 behavior in expanded visceral adipose tissue and the contribution of this tissue to circulating activity of these gelatinases.

Methods

Animals

Male Wistar rats ($n = 18$) obtained from the animal laboratory of the Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires (Argentina) were maintained under controlled temperature (22 ± 1 °C), humidity (50–60%), and air flow conditions, with a fixed 12-h light/dark cycle (light on7:00 AM to 7:00 PM). Until the beginning of the experiment, all animals were fed a standard rat laboratory chow and had free access to food and water to standardize their nutritional status. This diet provided approximately 2.9 kcal/g chow. When the rats' weight was 175–190 g, they were randomly divided into two groups: Control group ($n = 9$) and Sucrose rich diet (SRD) group ($n = 9$). Both continued to receive the preweighed standard diet, but the SRD group also received 30% sucrose in the drinking water throughout 12 weeks. In this period, the rats evolve to an early insulin resistance state [15,16], which was confirmed with insulin tolerance test (ITT) as previously described [19]. Body weight and caloric intake were monitored weekly throughout the experimental period. Possible dehydration was checked by plasma sodium measurement. All the procedures were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals [20] and the protocol was approved by the Local Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Samples

After 12 weeks of treatment, food and water were removed at the end of the dark period (7:00 AM). After 4 h of fasting, animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight). Blood samples were obtained and rapidly centrifuged at 1.500× *g* during 15 min; serum was kept at 4 °C within 48 h for the evaluation of glucose, lipids and lipoproteins or stored at −70 °C for further determination of insulin and free fatty acids (FFA). Plasma was obtained and stored at −70 °C for further MMP-2 and MMP-9 activity determination.

Epididymal, perirenal and intestinal fat tissue was removed and weighed in order to evaluate visceral adiposity. Epididymal adipose tissue (EAT) was fractioned and stored in liquid nitrogen for zymographic and Western blotting analysis and one sample was fixed in 4% formalin buffer, pH 7.0 and conserved at 4 °C for histological evaluation.

Assessment of body fat by X-ray absorptiometry

Body composition was assessed by dual energy X-ray absorptiometry (DXA) using a total body scanner Lunar DPX (DPX Alpha 8034, Lunar Radiation Corp., Madison, Wisc., USA), with software specifically designed for small animals. All DXA determinations were done by one investigator to avoid inter-assay error. Scans were analyzed by ultrahigh-resolution analysis software, and values for percent total fat DXA, which was the current interest, were recorded. The coefficient of variation was 2.2%.

Biochemical determinations

On the day of sacrifice serum glucose, total cholesterol and triglycerides (TG) levels were measured using commercial enzymatic kits (Roche Diagnostics GmbH, Mannheim, Germany) in a Cobas C-501 autoanalyser; the intra-assay CV was <1.9% and the inter-assay CV was <2.4% for all parameters. HDL-cholesterol was determined by standardized selective precipitation method using phosphotungstic acid/$MgCl_2$ as a precipitating reagent [21]. Given the naturally low plasma concentration of LDL-cholesterol in rats, no HDL-cholesterol was calculated as the difference between total-cholesterol and HDL-cholesterol as approximation of atherogenic lipoprotein levels.

FFAs were determined by a spectrophotometric method (Randox, UK), and insulin was measured with a sandwich ELISA kit using a monoclonal antibody against rat insulin and an enzyme-linked polyclonal antibody (Rat/Mouse