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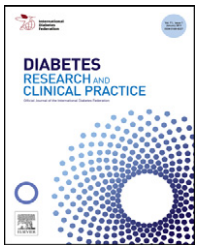

Changes in the arginine methylation of organ proteins during the development of diabetes mellitus

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ABSTRACT

Aim: In this study, we examined changes in asymmetric dimethylarginine (ADMA), dimethylarginine dimethylaminohydrolase (DDAH), nitric oxide synthesis (NOS), and the arginine methylation of organ proteins during the development of diabetes in mice.

Methods: Db/db mice developed significant obesity and fasting hyperglycemia during diabetogenesis. During diabetogenesis, the expression of ADMA and nNOS was increased, while that of DDAH1 and protein-arginine methyltransferase 1 (PRMT1) was decreased. Additionally, arginine methylation in the liver and adipose tissue was altered during diabetogenesis.

Results: Changes were evident at 75, 60, and 52 kDa in liver tissue and at 38 and 25 kDa in adipose tissue. Collectively, DDAH and ADMA are closely associated with the development of obesity and diabetes, and the arginine methylation levels of certain proteins were changed during diabetes development.

Conclusion: Protein arginine methylation plays a role in the pathogenesis of diabetes.

1. Introduction

An essential component of cell survival is protein degradation, which is a major source of intracellular and plasma arginine and methylarginine. Several conditions are associated with enhanced protein catabolism. For example, increased protein degradation has been reported in diabetes mellitus (DM). Obesity, insulin resistance, and endothelial dysfunction are closely associated with the development of type 2 diabetes. Diabetes is associated with several physiological abnormalities. Asymmetric dimethylarginine (ADMA) is a novel risk factor for type 2 diabetes that inhibits nitric oxide synthase (NOS) and causes endothelial dysfunction. Several metabolic syndromes contribute to endothelial dysfunction; the mechanisms of diabetes-induced endothelial dysfunction include the production of prostanoid vasoconstrictors and increased oxidative degradation of NO [1]. Endothelium-derived NOS regulates blood flow to insulin-sensitive tissues (e.g., skeletal muscle, liver, and adipose tissue), and its activity is impaired in insulin-resistant individuals [2]. Adipose tissue-derived factors may causally underlie these associations. ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) [3] extensively in the kidneys and liver, which are principal sites of DDAH1 expression. The ADMA/DDAH axis in adipose tissue may explain the association between insulin resistance and endothelial dysfunction in obesity.

We previously proposed that DDAH and ADMA are closely associated with the development of obesity and diabetes, and

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that arginine methylation plays a role in their pathogenesis. Additionally, ADMA, DDAH, and the arginine methylation of organ proteins likely change during diabetogenesis; indeed, decreased DDAH expression is the cause of increased ADMA. In this context, in the present study we examined the profile of DDAH expression and arginine methylation of organ proteins during the development of diabetes in diabetic and normal mice, and we assessed the role of arginine methylation in the pathology of diabetes by identifying the DDAH expression level and arginine methylated proteins.

2. Materials and methods

2.1. Metabolic studies

Thirty-six male mice were used. The experimental group ($n = 18$) consisted of homozygous type 2 diabetic mice (C57BLKS/J Iar −+Lepr db/+Lepr db); the control group ($n = 18$) consisted of misty, non-diabetic mice (C57BLKS/J Iar −m+/m+).

Db/db mice were first identified in 1996 as a model of obesity [4]. The diabetic gene (*db*) is an autosomal recessive mutation located on mouse Chromosome 4 that arose spontaneously in the C57BL/KsJ (BL/Ks) strain. The *db* gene encodes a G-to-T point mutation in leptin receptor, leading to abnormal splicing and defective signaling of the adipocyte-derived hormone leptin [5]. A lack of leptin signaling in the hypothalamus leads to persistent hyperphagia and obesity with consequent high leptin and insulin levels. Diabetes in C57BL/KsJ (db/db) mice is initially expressed as hyperinsulinemia, followed by hyperphagia, progressive obesity, and widespread pathologic abnormalities.

Due to possible crossover of the *misty* (*m*) and *Leprdb* genes, a low percentage of db/db mice could be heterozygous for the *m* gene.

We characterized the plasma glucose and related ADMA and DDAH levels in diabetic (db/db) mice and non-diabetic controls (misty) using strain C57BLKs/J. At weeks 5, 7, and 9, body weight was tested, and the plasma glucose level was evaluated by venous fasting blood sampling. The mice were then killed by CO_2 gas inhalation; blood was collected for measurement of the ADMA levels (in μmol/L); kidney, liver, and adipose tissue was collected for the determination of ADMA levels (in μmol/L); DDAH and NOS expression was assessed; changes in the level of asymmetric dimethylated proteins was examined; and protein-arginine methyltransferase (PRMT) expression was evaluated. Image analysis was performed using progenesis software.

2.2. Determination of the ADMA concentration

The concentration of ADMA was measured spectrophotometrically using an ADMA Direct (mouse/rat) ELISA Kit (Immundiagnostik, Bensheim, Germany) as per the manufacturer's instructions.

2.3. Western blot analysis

The densities of DDAH, NOS, arginine methylated protein, and PRMT1 were measured by Western blotting as follows. Samples were subjected to sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) using a Mini-PROTEAN 3 Cell System (Bio-Rad), followed by transfer to a polyvinylidene fluoride (PVDF) membrane (Millipore) using a semi-dry transfer cell system (Bio-Rad; 25 V for 60 min). Thereafter, the membrane was immersed in methanol for about 10 s and blocked by drying in air. The membrane was incubated with the first antibody (1:1000 in 3% skim milk) overnight at 4 °C. Thereafter, the membrane was washed with Tris-buffered saline with Tween 20 (TBS–T) twice for 5 min each, followed by incubation with the second antibody (1:1000 in 3% skim milk) for 1 h at 4 °C. The membrane was then washed with TBS–T twice for 10 min each, followed by incubation with horseradish peroxidase substrate peroxide solution (1:1) and exposure to X-ray film.

2.4. Determination of the arginine methylated protein and PRMT1 levels

To identify dimethylated arginine-containing cellular proteins and PRMT1, immunoprecipitations were performed using asymmetric 24 (ASYM24). ASYM24 was generated using the peptide KGR*GR*GR*GR*GPPPPPR*GR*GR*GR*G (in which R* denotes the asymmetric dimethylation of arginine) as an antigen. ASYM24 recognizes ADMA specifically and was shown to recognize Sam68, an RNA-binding protein [6]. The epitope for ASYM24 is significantly diminished in PRMT1−/− cells, demonstrating that the major enzyme that contributes to the ASYM24 epitope is PRMT1 [6]. A control immunoprecipitation was performed using glyceraldehyde-3-phosphate dehydrogenase.

2.5. Statistical analysis

Our results are expressed as the mean ± standard error of the mean. Comparisons between groups were made by Students' *t*-test for unpaired observations with SPSS version 12 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. An increase in body weight and fasting plasma glucose levels is associated with DM

In the C57BLKs/J db/db mice, hyperinsulinemia was observed by 10 days of age; blood glucose levels were slightly elevated at 1 month of age. After 1 month of age, the db/db mice could be distinguished from wild-type and heterozygous mice by the presence of increased fat deposition in the inguinal and axillary regions. A progressive increase in food and water intake was found to be associated with progressive weight gain until 4–5 months of age [7]. After 5–6 months of age, body weight and insulin levels begin to fall in association with pancreatic islet cell degradation [8]. At this time, the mice become so obese that they have difficulty ambulating in the cage and obtaining food and water. In our study, within 5 weeks of age, the db/db mice developed significant obesity and fasting hyperglycemia ($P < 0.05$). Thus, db/db mice are suitable