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Long-term dietary sodium restriction increases adiponectin expression and ameliorates the proinflammatory adipokine profile in obesity

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Abstract *Background/aim:* Obesity is associated with changes in adiponectin and pro-inflammatory adipokines. Sodium intake can affect adipokine secretion suggesting a role in cardiovascular dysfunction. We tested if long-term dietary sodium restriction modifies the expression of adiponectin and ameliorates the pro-inflammatory profile of obese, diabetic mice.
Methods/results: Db/db mice were randomized to high sodium (HS 1.6% Na+, $n = 6$) or low sodium (LS 0.03% Na+, $n = 8$) diet for 16 weeks and compared with lean, *db/+* mice on HS diet ($n = 8$). Insulin levels were 50% lower in the *db/db* mice on LS diet when compared with HS *db/db* ($p < 0.05$). LS diet increased cardiac adiponectin mRNA levels in *db/db* mice by 5-fold when compared with *db/db* mice on HS diet and by 2-fold when compared with HS lean mice (both $p < 0.01$). LS diet increased adiponectin in adipose tissue compared with *db/db* mice on HS diet, achieving levels similar to those of lean mice. MCP-1, IL-6 and TNF-α expression were reduced more than 50% in adipose tissue of *db/db* mice on LS diet when compared with HS *db/db* mice (all $p < 0.05$), to levels observed in the HS lean mice. Further, LS *db/db* mice had significantly reduced circulating MCP-1 and IL-6 levels when compared with HS *db/db* mice (both $p < 0.01$).

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Conclusion: In obese-diabetic mice, long-term LS diet increases adiponectin in heart and adipose tissue and reduces pro-inflammatory factors in adipose tissue and plasma. These additive mechanisms may contribute to the potential cardioprotective benefits of LS diet in obesity-related metabolic disorders.

Introduction

Obesity is associated with increased prevalence of type 2 diabetes (T2DM), hypertension, metabolic syndrome (MetS) and cardiovascular disease (CVD). Human and rodent obesity are characterized by reduced adiponectin levels and elevated levels of proinflammatory cytokines, which both contribute to the development of associated metabolic disorders [1]. According to a recent-meta-analysis, lower adiponectin levels are amongst the strongest biochemical predictors of insulin resistance and T2DM [2]. Interestingly, adiponectin is not only secreted by adipocytes but also is produced by cardiomyocytes and several studies report a cardioprotective effect of adiponectin on hypertension, heart failure and endothelial dysfunction [3,4].

Obesity-related inflammation and cardiometabolic disorders have been associated with increased aldosterone levels and excess mineralocorticoid receptor (MR) activation [5]. We showed previously that MR blockade with eplerenone increases adiponectin and reduces inflammatory cytokines in obese *db*/*db* mice [6]. Studies examining the adverse cardiovascular effects of MR demonstrate an important influence of dietary sodium intake—a high sodium diet increases and a low sodium diet reduces MR-mediated cardiovascular and renal damage [7—10]. In addition, an animal model of aldosterone-induced injury showed that treatment with an MR antagonist or dietary sodium restriction has similar cardioprotective effects [9]. To date there is sparse and controversial information regarding a potential protective effect of low sodium diet on the obesity-related pro-inflammatory adipokine profile [4,11].

The aim of the present study was to test the hypothesis that long-term dietary sodium restriction improves the expression of adiponectin in heart and adipose tissue, and reduces inflammatory cytokines of obese, diabetic *db*/*db* mice.

Methods

Animal protocol

We studied three groups of male rodents with dietary sodium intervention for 16 weeks from age 9 weeks until age 25 weeks. We selected lean, *db*/+ littermates on high sodium (HS) diet as a control group since high sodium intake is the most frequent diet worldwide.

The study group design was as follows: 1) lean, non-diabetic control group *db*/+ mice (HS, 1.6% NaCl, $n = 8$); and obese, diabetic *db*/*db* mice randomized to either 2) a high sodium diet (HS, 1.6% NaCl, $n = 6$), or 3) a low sodium diet (LS, 0.03% NaCl, $n = 8$). The obese *db*/*db* mice (Jackson Laboratory, Bar Harbor, ME) are generated by mating male and female db/+ mice and thus are littermates with similar genetic backgrounds except for the number of mutated leptin receptors [12].

To confirm appropriate sodium intake, mice were placed in metabolic cages for 24 h urinary sodium determination, considered the gold standard measurement [13]. Systolic blood pressure was measured in conscious animals by tail-cuff plethysmography at age 25 weeks (Blood Pressure Analyzer, Model 179, IITC Life Science). Animals were kept in a room lighted 12 h/day at an ambient temperature of 22 ± 1 °C. At 25 weeks of age, blood samples, visceral adipose tissue from the retroperitoneum, and hearts were harvested. The Institutional Animal Care and Use Committee at Harvard University approved experimental procedures.

Biochemical parameters

Plasma aldosterone levels and plasma renin activity (PRA) were determined using radioimmunoassay techniques as previously described [14].

Plasma glucose was measured using Cobas Integra 400 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) via a hexokinase enzymatic reaction. Plasma insulin was measured using the LincoPlex mouse insulin ELISA assay (LINCO Research, St. Charles, MO). To estimate insulin resistance, the homeostatic model assessment (HOMA) index was calculated. This method, despite its limitation compared to euglycemic glucose clamp, has been validated in rodents [15].

Plasma cytokine concentrations

Cytokine protein concentrations of interleukin (IL)-6, IL-10, monocyte chemotactic protein-1 (MCP-1), interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) were measured in four animals per group using the Cytometric Bead Array System (mouse inflammation CBA kit, Cat #552364, BD Biosciences, San Jose, CA). Cytokine concentrations were determined by flow cytometry (BD FACScan, BD Biosciences). Results take into account the total protein concentration of the plasma and were expressed as pg/ml. Intra-assay variability was 2% and inter-assay variability was 5%.

Quantitative real-time PCR

Total mRNA was extracted from visceral adipose tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen Sciences, Germantown, MD) and from heart tissue using the RNeasy Mini Kit (Qiagen Sciences). PCR amplification reactions